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14. ABSTRACT This study has been designed to evaluation the sequestration of proprietary radiopharmaceuticals Radioactive Galium Iron Macroaggregates (GIMA) after intratumoral injection. Our team has adopted a 2-prong approach. While the official approval of the human protocol is ongoing, we continue on translation studies using in vivo imaging methods to investigate our institution-approved animal tumor models in rats and dogs. We made revisions the approved human protocol and received preliminary approval from the Army in March 2005, awaiting the final approval of the revisions by the MDACC IRB. Because of the de-commission of our institutional Radioactive Drug Research Committee (RDRC) in 2005, we have sought other approval avenues to conduct the human study in compliance with FDA regulation. While an IND route was in progress, our large animal tumor models in 5 dogs (>20Kg) yielded confirmatory results for sequestration of Ga-68 GIMA. The remaining scientific question is the demonstration of persistent sequestration of GIMA in human breast cancer for derivation of dosimetry. In the meantime, our institution has reapplied to the FDA to reestablish the RDRC, which will be the most appropriate authority to supervise the low-dose Ga-67 GIMA protocol of 5 patients. We expect to regain the RDRC approval within the next few months and conclude this project within 12 months.

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Introduction

This study is to demonstrate the sequestration of our propriety radioactive Gallium Iron Macroaggregates (GIMA) in human solid tumors, using breast cancer as a starting point. We have designed and revised the single IRB-approved protocol (ID03-0070) into 2, one for each radiopharmaceuticals of Ga-67 GIMA and Ga-68 GIMA to study the short-term (hours) sequestration and long-term (days) sequestration, respectively. Both revised protocols (2005-0219 and 2005-0220 in Appendice II and III) have gained the preliminary approval from the Army Human Subject Protection Committee, pending final approval from our IRB. However, developments within our institution in 2005 rendered the Radioactive Drug Research Committee inactive. We have also worked with the institutional research administration to pursue the IND.

In the meantime, we continued our translation research studies to support future clinical applications. This year, our concentration is on the study of intratumoral Ga-68 GIMA sequestration in tumors of large animals. A dog canine transmissible venereal tumor (cTVT) was chosen because of the large size (25-35 Kg) of the dog to approximate the human proportions. Large animal can also tolerate the effects of tumor growth to larger size. Both lung tumor and prostate tumor models were chosen. After gaining approval from institutional animal care and utilization committee (IACUC), we used PET/CT and MRI to study Ga-68 GIMA distribution after intratumoral injection into implanted lung tumors and prostate tumors because breast tumor is not available in this model. The measured standard uptake values (SUV) in the tumors measuring between 1500 to 4200, confirming the sequestration of GIMA after injection. There is also persistence of this sequestration over several hours which reached temporal limits to study Ga-68 with a half-life of 1 hour. However, the long-term persistent sequestration necessary for effective radionuclide therapy has not been demonstrated and requires the use of a radioactive gallium with longer half-life (e.g., Ga-67 with 3 day half-life). Since we have demonstrated Ga-68 GIMA intratumoral sequestration in large animals, we have chosen to study on Ga-67 GIMA in humans, pending the final approval of the protocol 2005-0219. When the feasibility of using Gallium Iron Macroaggregates (GIMA) in human breast cancer is confirmed by the clinical studies, our translation research will also guide the application of GIMA in other human solid tumors.

Body

1) Revision of our IRB-approved protocol ID03-0070 into 1 protocol (2005-0219) which has gained preliminary approval from the Army Human Subject Protection Committee, pending our IRB approval. Re-application for approval under our institutional overseeing Radioactive Drug Research Committee which is expected to be re-established by September 2006. (Appendix I, re-submitted 6/2006)

2) Institutional Approval of cTVT dog tumor models of lung and prostate tumors (ICUAC-approved protocol in Appendix II)

3) Scintigraphic studies of Ga-67 GIMA in rats and PET/CT studies of Ga-68 GIMA in dogs (Appendix III, Reference 1).

3) After resignation of Andres Fonnegra DDS in March, we recruited a new of Research Coordinator Farrah Chickerneo, MD (CV in Appendix IV).

Key Research Accomplishments

- Confirmation of sequestration of Ga-68 GIMA after intratumoral injection in larger tumors (2-10cc) in dog tumor models of prostate and lung tumor
- Demonstration of metabolic suppression of glucose metabolism in dog lung tumor by intratumoral injection of Ga-68 GIMA
- Demonstration of histopathologic changes inside lung tumors after intratumoral injection dog Ga-68 GIMA
- Revisions and re-submission of Protocol 2005-0219 to MDACC for approval on 6/30/2006.

Reportable Outcomes

Presentations:

November 2005, International Animal Molecular Imaging Symposium, Taiwan, China

Wong, F. C. Nuclear Imaging: From Rats to Dogs to Humans.

Conclusions

We are proceeding with our 2-prong approach with different degree of success. The development of the human protocol for the conformance with regulatory requirements is still ongoing, while we are awaiting the re-establishment of our overseeing institutional committee (expected in 9/2006). Our preparatory animal studies have been expanded from rats into larger animals to provide good approximation to human size for better imaging resolution and for dosimetry. Our tumor models in dogs have yield confirmatory findings for the sequestration of Ga-68 GIMA after intratumoral injection for a few hours. The long-term sequestration is still to be studied with Ga-67 GIMA to derive the dosimetry. Once our human study confirms the feasibility of locoregional radionuclide treatment in the breast, similar strategies can be designed for other human solid tumors, with the insights from the animal studies.

References

Wong, F. C. Nuclear Imaging: From Rats to dogs to Humans, In: International Animal Molecular Imaging Symposium, Ch. 5, p35-40, Elsevier, 2006.

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

Protocol Page

Radiation Dosimetry from Intratumoral Injection of Radionuclides into Human Breast Cancer Ga 67 GIMA
2005-0219

Core Protocol Information

Short Title	Intratumoral Injection of Radionuclides (Ga 67 GIMA) into Human Breast Cancer
Study Chair:	Gary Whitman
Additional Contact:	Franklin Wong
Department:	Nuclear Medicine
Phone:	713-794-4649
Unit:	59
Full Title:	Radiation Dosimetry from Intratumoral Injection of Radionuclides into Human Breast Cancer Ga 67 GIMA
Protocol Type:	Standard Protocol
Protocol Phase:	N/A
Version Status:	Submitted
Version:	00
Submitted by:	Gary Whitman--6/30/2006 1:55:53 PM

Protocol Body

1.0 Objectives

Select Section Title: 1.0 Objectives

The Objectives are:

1. Use MRI to measure the spatial and temporal profiles of GIMA after intratumoral injection into breast cancer

2. Use high resolution gamma scintigraphy for Ga-67 GIMA to measure the spatial and temporal profiles of the radioactivity of GIMA after intratumoral injection into breast cancer;

3. Use the imaging data from MRI and nuclear imaging to calculate whole-body, organ, and locoregional radiation dosimetry to evaluate safety and efficacy factors for intratumoral GIMA.

The Hypotheses are:

1. After intratumoral injection, GIMA will be dispersed but remain contained in the tumor.
2. The radiation absorbed doses will be high within the tumor but low in the body and surrounding organs.

2.0 Background

Select Section Title: 2.0 Background

Locoregional Radiation Therapy of Breast Cancer - a beginning

Multiple trials of breast conservation in patients treated with and without whole breast radiation have found that the majority (> 90%) of local recurrences occur at the site of surgical resection [1]. Clinical trials have confirmed the usefulness of sealed radionuclides as internal radiation sources for locoregional adjuvant treatment of breast cancer, as demonstrated by the recent FDA approval of MammoSite using Iridium-192 [2]. Therefore, conventional radiation treatment to the whole breast following breast conserving surgery may not be a necessary approach for the majority of women. More directed local treatment with radiotherapy appears to be safe and effective treatment. Conventional brachytherapy involves the implanting of sealed radiation sources implanted into the post-surgical field for several weeks [3, 4]. Recent clinical trials have reported favorable outcomes treating brain and breast cancer patients using a single implanted catheter filled with Iodine-125 Iotrex and Iridium-192 seeds irradiating the tissues around the post-surgical cavity (by Proxima Therapeutics, Inc.). This approach has recently gained FDA approval (GlialSite for brain tumor and MammoSite for breast cancer [1, 5, 6, 7]). Locoregional radionuclide therapy offers several desirable features: predictable dosimetry, the capability of being monitored, and short duration. Ablating breast tumors using intratumoral injection of radionuclides without sealing (e.g. by a catheter) has not been explored. This is due to the lack of requisite information on radionuclide dispersion and on radiation dosimetry in the tumor and surrounding tissues to establish efficacy and safety. This proposed study aims to explore the feasibility of using intratumoral injection of unsealed radionuclides as internal

radiation sources.

Breast Lymphoscintigraphy - an opportunity to study radionuclides in human tissues

Breast lymphoscintigraphy is a nuclear medicine procedure that is increasingly important in the identification of sentinel lymph node(s). Typically, aliquot(s) of about 1cc containing 0.5 mCi of Technetium-99m (Tc-99m) labeled sulfur colloid (SC) is injected percutaneously into the tumor or breast tissues around the tumor. Smaller sizes (<0.22 micron) of SC allow better lymphatic drainage and therefore better visualization of the sentinel lymph node(s). Only a small fraction (<1%) [8, 9] of the SC injected ever drains via the lymphatics to allow visualization of the sentinel lymph node(s) . Conversely, particles of larger sizes (>0.22 micron) or direct intratumoral (IT) injection of SC into the breast tumor reveals even less lymphatic drainage. Although unsealed, radionuclides injected into the tumor or surrounding tissues are indeed subject to spatial sequestration. The injection site appears spherical and unchanged (for days) on scintigrams. Although difficult to quantify, ultrasound guidance during selected breast lymphoscintigraphy shows that injections of SC into the breast tissue result in a larger dispersed volume which has not been adequately assessed. Radiation dosimetry of breast lymphoscintigraphy have shown variations up to ten-fold [10, 11, 12], partly because of the imprecision in determining the volume of the dispersed injectate. An injection of 0.5 mCi Tc99m SC delivers about 40 cGy to the injection site and 4 cGy to the sentinel lymph node. When standard guidelines are observed, there is good margin for radiation safety and the radiation absorbed dose to the sentinel lymph node is about one tenth that of the injection site [13]. The Medical Internal Radionuclide Dosimetry (MIRD) schemes require accurate determination of volume and residence time of dispersed radionuclides [14]. A recent report directly measured the injectate volume using the full-width half maximum (FWHM) of the injection site from the scintigram. The accuracy of this volume estimate was limited by the system resolution of 2 cm [12]. The search for an accurate measurement of the dispersed injectate volume for dosimetry has been futile because, besides the radioactivity, there is no other physical signal from the injected radionuclide for external imaging.

A paramagnetic radiopharmaceutical Gallium-Iron Macroaggregate (GIMA) has been identified to provide both radioactive and paramagnetic signals for external measurement. This study is designed to evaluate the volume of dispersion and radiation dosimetry of GIMA after intratumoral injection into untreated human breast tumor.

Radionuclide Dosimetry of Unsealed Sources- Simulated Radiation doses to tumor and surrounding tissues

Earlier general internal dosimetry schemes including MIRDose3 (an established Medical Internal Radiation Dosimetry program) do not provide depth dosimetry to account for surrounding tissues. Earlier reports of simulation are limited to specific radionuclides in specific configurations [15, 16]. In our study, Monte Carlo simulation for Y-90 Zevalin was applied and found helpful in defining regions of toxicity [17]. A simulation project using sphere and shell models with common core volumes of 0.4, 2, 10, 50 and 250 cc is continuing and we reported radiation dosimetry in the core and 30 concentric layers from 19 radionuclides [18]. As predicted before, the radiation absorbed doses to the sentinel lymph nodes will be about one

tenth of those to the injection sites in the tumor. The extremes of heterogeneous distribution of radionuclides in the lesion were reported using shell models assuming that all the radionuclide was confined to the first layer around the central cavity. There was little dosimetry difference from the sphere models (<10%) in tissues beyond 1 cm. These sphere [18] and shell models [19] provide estimates of dosimetry ranges. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. This group (0.2 mCi Ga-67 GIMA) of 5 patients will receive estimated doses of 463cGy in the injection site, with a 10% isodose range of 0.02cm from the injection site edge. Based on preclinical studies suggesting a total of 2% leakage of radiogallium in the form of free Ga(+3), the MIRDose3 models predict low radiation absorbed doses to the vital organs in units of cGy/mCi:

SIMULATED DOSIMETRY	**Ga-67 GIMA**	**0.2 Mcl Ga-67 GIMA for this study**
ORGAN	**Rad/mCi**	**Total rads**
Adrenals	0.1200	0.0240
Brain	0.0140	0.0028
Breast w/ Injectate	22.0000	4.4000
Breast wo/Injectate	0.5900	0.1180
Gallblader Wall	0.0940	0.0188
LLI Wall	0.0290	0.0058
Small Intestine	0.0270	0.0054
Stomach	0.1500	0.0300
ULI Wall	0.0450	0.0090
Heart Wall	0.5900	0.1180
Kidneys	0.0590	0.0118
Liver	0.1700	0.0340
Lungs	0.4500	0.0900
Muscle	0.1000	0.0200
Ovaries	0.0150	0.0030
Pancreas	0.1400	0.0280
Red Marrow	0.1200	0.0240
Bone Surfaces	0.1700	0.0340
Skin	0.1600	0.0320
Spleen	0.1100	0.0220
Testes	0.0053	0.0011
Thymus	0.5800	0.1160
Thyroid	0.0790	0.0158
Urine Bladder Wall	0.0140	0.0028
Uterus	0.0170	0.0034
Total Body	0.3200	0.0640
EFF DOSE EQUIV	5.1000	1.0200
EFF DOSE	1.9000	0.3800

Using human breast tumor as a model system, dosimetric measurement will be achieved by acquiring the spatial and temporal distribution of injected GIMA, measured from MRI and nuclear imaging. Ga-67 GIMA (physical half-life of 78 hours) is used to measure the prolonged distribution of radioactivity using a gamma camera. Confirmation of the sequestration and derivation of radiation dosimetry will permit variations to achieve high radiation dose for therapeutic effects. For instance, larger amounts of radioactivities may be achieved by using larger volumes of GIMA while maintaining the Ga/Fe ratio; alternatively, larger radioactivities may be delivered by increasing the Ga/Fe ratio while maintaining the volume of the injectate. Results from this dosimetric study will provide bases for the design of future phase I and II clinical trials to use this class of radiopharmaceuticals to treat selected subgroups of patients with breast cancers and to correlate with biologic markers.

3.0 Background Drug Information

Select Section Title: 3.0 Background Drug Information

A known radiopharmaceutical Ga-68 /Fe macroaggregates (GIMA) [20] that may provide paramagnetic signals for volume measurement by MR imaging and simultaneously emit gamma rays for nuclear imaging was identified . It has a biologic half-life of 30 hours, a physical half-life of 1.1 hours and measures 10-30 micron in size. It was used in human lung perfusion imaging in the 1970's until the advent of the current imaging agent of Tc-99m -macroalbumin aggregates. It was produced in a carrier-added (additional nonradioactive gallium) preparation (0.12 Ci/mole) containing large amounts of additional nonradioactive gallium which in turn caused dose-limiting toxicity [20]. Following similar steps while deleting the toxic nonradioactive gallium (carrier), our laboratory has managed to produce carrier-free GIMA (with Ga-68 and Ga-67, respectively) of good stability (>98% after incubation in PBS for 24 hours) and confirmed the large sizes (99%>0.5 microns). Additionally, we have demonstrated decreases in Gradient Echo (GRE) signals on MRI with increasing Fe contents in the concentration range intended for intratumoral injection. Ga-67 GIMA is a gamma-ray emitter with a physical half-life of 78 hours during which the long-term organ distribution of GIMA can be monitored using gamma-cameras.

The dry density of Iron Macroaggregates is about 2.66 gm/cc. However, with only 1 mg in the 1 cc solution, the density of the solution is only slightly higher than 1.0 gm/cc. With periodic shaking before injection, our team had no difficulty during injection in animal into small size tumors; we do not expect difficulty injecting into humans.

3.1 Supplier/How Supplied

3.1.1 Carrier-free Gallium-67 GIMA will be prepared according to the method of Colombetti [20] with the exception that commercially available radiopharmaceutical grade Ga-67 (nominal specific activities >30 Ci/mmole because of non-carrier added preparations) will be used and no non-radioactive gallium (carrier) will be added. The starting materials also involve ultra-high grade of iron chloride ((Iron (III) Chloride, anhydrous, powder, 99.99+% LOT # 04134TB SIGMA-ALDRICH, Inc, 3050 Spruce St. St Louis, MO 63103 USA.) and 0.22um-filtered sterilzed PBS buffer and ammonia (Ammonium hydroxide, 28% NH3 in water,99.99% LOT# 07923LA SIGMA-ALDRICH, Inc, 3050

Spruce St Louis, MO 63103 USA). The final product (Ga-67 GIMA, synthesized according to Appendix A) is a colloid suspended in saline. Our previous experiments have consistently produced Ga-67 GIMA with >90% radiochemical yields. Only batches of >90% radiochemical yield will be used. Aseptic procedures will be followed and pyrogenicity test will be performed and negativity will be confirmed before injection. Waterproof gloves will be worn by the personnel during preparation procedures. Ga-67 GIMA will be stored in sterilized vials behind lead bricks.All vials will be brought to room temperature immediately prior to use. Part of the contents in the vial to be injected will be tested for the evaluation of pyrogenicity using the LAL assay (Whittaker Bioproducts, Walkersville, MD) which will last approximately 30 minutes. Unused vials or portions of Ga-67 products will be eliminated by nuclear decay in storage behind lead bricks for at least 4 weeks. The synthesis and testing procedures typically last 80 minutes. The suspended colloid is available in screw-cap vials with radioactivity ranging from 0.1 to 2 mCi per vial. The total iron content is approximately 2 milligrams. The sterility of the products will be tested and monitored for 10 days for aerobic and anaerobic pathogens using BD Bactec Plus/F and Thioglycolate cultures (Becton and Dickinson, Sparks, MD), as a standard testing procedure of radiopharmaceuticals of short half-lives.

3.1.2 Through a confidentiality disclosure agreement, the South Texas Nuclear Pharmacy has agreed to provide carrier-free Ga67-GIMA in pyrogen-free (LAL test-negative) conditions and monitor sterility tests for each dose preparation for 10 days. They will followed procedures outlined in Appendix A.

3.2 Determination of radioactivity of GIMA.

The radioactivity of Ga-67 GIMA total products and individual patient dose will be measured by a Capintec dose calibrator in the MDACC Nuclear Medicine Nuclear Pharmacy (with daily quality assurance check) and the volume will be noted along with the time of measurement.

3.3 Storage and Disposal

Ga-67 GIMA will be handled only by our Clinical and Scientific staff (Physician, Nurse, Chemist or Nuclear Medicine Technologist).Unopened vials of Ga-67 GIMA will be stored for decay at room temperature and shielded from sunlight behind lead blocks in the nuclear pharmacy storage vault. After their radiation level fell to background level, they will be disposed. It is expected Ga-67 will take up to 4 weeks.

3.4 Toxicity.

From the published results of human lung scanning, no adverse effects have been attributed to GIMA . Published toxicity of gallium compound has been correlated with the nonradioactive free gallium (carrier) with a limiting dose of 1mg, corresponding to the about 0.1 mCi of the low-specificity GIMA. At multi-milligram levels of systemic administration, the tyical symptoms include gastrointestinal discomfort and hepatic failure. The high specific activity GIMA prepared by our method contains no

nonradioactive gallium and the physical amount of gallium (Ga-67) is one-billionth that of the earlier preparation [20] and is therefore well below the toxicity threshold. In fact, cancer patients injected with larger systemic doses up to 10 mCi carrier-free Ga-67 (as used in routine tumor localization imaging) do not have signs of toxicity.

4.0 Patient Eligibility

Select Section Title: 4.0 Patient Eligibility

All Study patients must meet the eligibility criteria:

4.1 Eligible Patients

- 4.1.1 Patients must understand the procedures and the explanations in English and must provide informed consent by signing the informed consent form
- 4.1.2 Patients must be 18 years of age or older
- 4.1.3 Patients must have breast cancer diagnosed by histopathology but no surgical resection of the tumor.
- 4.1.4 Patients should have received no previous focal external beam radiation therapy to the thorax.
- 4.1.5 Patients who have not received systemic or cytotoxic chemotherapy for the breast cancer under study. Patient under hormonal therapy alone will be eligible.
- 4.1.6 Patients with adequate platelets to avoid excessive bleeding and adequate white cells to avoid infection.
  - Granulocytes >= 1000 cells/mcl
  - Platelets >=40,000/mcl
- 4.1.7 Patients with Zubrod performance scale of 2 or below.
- 4.1.8 Patients with breast tumor > 2 cm compressed thickness on mammogram but no tumor necrosis by MRI.
- 4.1.9 Patients must have scheduled surgical resection (either mastectomy or conservation surgery) of the breast tumor within 2 weeks after injection.
- 4.1.10 Patients with a F-18 FDG PET within 2 weeks showing a tumor SUV uptake > 2.0

4.2 Ineligible Patients

- 4.2.1 Patients of child-bearing potential (not post-menopausal for 12-24 months or not surgical sterile)who have positive pregnancy test or are lactating.
- 4.2.2 Patients with septicemia, severe infection or acute hepatitis.
- 4.2.3 Patients who had radiation therapy or chemotherapy of the breast cancer prior to the planned surgery.
- 4.2.4 Patients who had residual radiation from previous radionuclide administration, from the day of injection:
  - F-18 agents of more than 10 mCi within 2 days.
  - In-111, Ga-67 or I-131 agents of more than 1 mCi within 14 days.
- 4.2.5 Patients who cannot undergo MRI procedures (including nonvisualization of tumor on MRI and implants incompatible with MRI)
- 4.2.6 Patients with claustrophobia cannot be entered for the Ga-67 GIMA groups because of the requirements of repeated MRI requiring repeated conscious

sedation.

4.2.7 Patients who have scheduled surgical resection of the breast tumor in less than 7 days are not eligible to enter the Ga-67 GIMA groups

4.2.8 Patient who cannot understand the procedures as explained in English or who cannot provide meaningful informed consent.

5.0 Treatment Plan

Select Section Title: 5.0 Investigational Plan

Despite the title, this section is indeed an Investigational Plan.
Breast cancer is predominantly a disease of the adults and only patients above 18 year old will be eligible to enroll. No dose adjustment is made for younger patients because of statistical requirements of uniformity for small sample sizes and because GIMA is expected to stay only within the tumor.

5.0.1 Subject Identification

A unique patient research ID number will be assigned to each individual participating the study. The subject ID will consist of 5 digits in the format of GG-NNN where GG is the group ID for the institution and NNN is the accession number within the institution. The unique patient research ID number will be assigned by the study PI. A password protected secured file will be created to store the cross reference list between the patient research ID number and confidential patient information such as name, birth date, hospital number, and social security number (if available), etc. Patient research ID number will be used throughout the trial and in database for patient identification purpose. Confidential patient information will be used only when it is necessary such as in patient care setting.

5.0.2 Ethical and Legal Considerations

This study will undergo full approval in accordance with the human surveillance requirements of our institution. Blood and urine samples will be obtained for the evaluations as described in the protocol. Measures will be taken to ensure confidentiality of participant information. Data collected on paper forms will be stored in locked file cabinets with restricted access. Data collected on electronic media will be stored in computer files with restricted password access. All staff members in the study will be informed prior to employment and at regular intervals of the necessity for keeping all data confidential. Computers will not be accessible to the public and will be located in locked offices. Subjects will be assigned a separate study number to protect subject identification. No patient identifiers will be used in any publications of this research. Data will be maintained indefinitely and representatives of the United States Army Medical Research and Materiel Command may inspect research records. When the time comes to dispose of the data, all database files will be deleted.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) of this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. Gary Whitman is the principal investigator who will supervise this study in UTMDACC. Either Dr.Gary Whitman or Dr. Mark Dryden will perform the injection of Ga-67 GIMA.

5.0.3 Division of Responsibilities

5.0.3.0 Study Personnel

A description with roles and responsibilities of the study personnel, as well as , contact information can be found in appendix K.

5.0.3.1 Medical Monitor

For this protocol Dr. Richard Theriault has been designated medical monitor. Dr. Theriault is a qualified physician, other than the principal investigator, not associated with the protocol, able to provide medical care to research volunteers for conditions that may arise during the conduct of the study, and who will monitor the volunteers during the conduct of the study. Dr. Theriault will review serious adverse events and unanticipated problems.

Dr. Theriault will review all unanticipated problems involving risk to volunteers or others, serious adverse events and all volunteer deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the medical monitor should and will comment on the outcomes of the event or problem, and in the case of a serious adverse event or death, comment on the relationship to participation in the study. The medical monitor should and will indicate whether he/she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or medical monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the Human Subjects Research Review Board (HSRRB).

5.1 Human Study:

Patients will be recruited from female breast cancer patients scheduled for surgery at least one-week from the planned day of injection. One of the inclusion criteria will be a tumor size of > 2cm in diameter but no tumor necrosis as determined by MRI. No spillage outside of the tumor is expected from an injection of 1 cc. The MRI and nuclear imaging studies will follow routine clinical procedure.

This group will receive 0.2 mCi intratumoral injections of Ga-67 GIMA (in 1cc saline) under MRI guidance and then undergo whole-body scintigraphy, MRI and

ultrasound at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Blood sample (2-5 cc each) collection will take place during each of the scintigraphy session. Although the exact radiation dosimetry has yet to be determined, the radiation doses to the tumor can be estimated from the published biological half-life of 30 hours [20]. This group of 5 patients will receive estimated doses of 262 cGy in the tumor, with a 10% isodose range of 0.02 cm from the injection site edge.

5.1.1 Patient Entry Requirements

Patients entered into this patient treatment study must meet eligibility requirements and sign the informed consent form. Each patient will be given a standard medical examination including a breast MRI with a medical history and laboratory work to determine eligibility. Patients will be accrued from the breast oncology clinics. When a patient with a breast cancer larger than 2 cm and who needs to undergo surgery is identified, the patient will be interviewed and provided information about this study. Signed informed consent will be obtained by Dr.Gary Whitman, Dr Mark Dryden or other physician investigators (except Dr. Franklin Wong because of potential conflict of interest) no earlier than the next day after the initial interview to provide adequate time for the patient to consider participation.

5.1.2 Informed Cosent Process

Referring physicians and clinic staff will be provided with flashcards (Appendix B) including eligibility criteria and contact information. After a patient is considered a candidate, the referring physician will explain the study and give the patient a Recruitment Information Brochure (Appendix C). Upon patient's contact with our research coordinator or protocol chairman, the eligibility criteria will be reviewed. If found eligible, a physician investigator (Dr. Gary Whitman, Dr. Mark Dryden or others) will interview the patient and provide further information of the study and answer relevant questions before obtaining informed consent as evidenced by patient's signing on the current version of IRB-approved informed consent form. Then the patient will be given a Study Procedure Instructions Flyer (Appendix E) which further explain the details of the procedures.

A statement reading :" There is no direct benefit to the patient from this investigational study" will be included in the informed consent form.

5.2 MRI and Injection

The patients will be scanned using a GE Signa Lx 1.5 Tesla MRI scanner equipped with a high performance gradient system (amplitude = 22mT/m; slew rate = 120 T/m/s). A phased-array bilateral breast RF coil will be used to maximize the signal-to-noise ratio. A breast positioning system with two compression plates and Vitamin E markers will be used to hold the breast in a reproducible position. Image slice thickness will be approximately 6mm and the scan will be positioned to also include the axilla as much as possible.

Before entry into the study, patients will have been asked about metallic implants

such as pacemakers and other devices. Patient with these conditions will have been excluded as determined by the MRI radiologist collaborators. Specificial and easily accessible tumors will be preferred over other locations. An MR-compatible 20-gauge needle of 7 cm in length will be used. If requested, ear plugs will be provided to decrease noise during MRI.

The breast tumor will first be localized using a fast gradient echo T1-weighted 3D pulse sequence in the sagittal plane. An MR-compatible disposable sterile needle will be placed intra-tumorally. Areas of necrosis, if any (developed after the pre-injection MRI), will be avoided. An MR scan will be performed to ensure the proper location of the needle. Prior to injection, a high-resolution baseline image will be obtained using a gradient echo (GRE) pulse sequence with parameters selected to be sensitive to T2*. Injection may be preceded with surface or subcutaneous local anesthesia (1cc of 4% lidocaine, if the patient has no history of allergy or adverse reactions to lidocaine; otherwise, an alternative local anesthetics will be used) at the needle entrance through the surface. The GIMA will be injected in one single intratumoral injection into the tumor over 1 minute. The needles will then be slowly removed. This procedure is similar to routine breast lymphoscintigraphy . Immediately after injection, images (without breast compression) from multi-phase T2*-weighted MRI will be acquired using the same pulse sequence in quick successions up to 1 hour. All subsequent MRI images will be without breast compression. The volume of the injectate will be determined from manual segmentation.

5.3 Nuclear imaging using High Resolution Scintigraphy

After MR guided injection and imaging, the patient will be sent to the Nuclear Medicine Clinic. The radioactivity residence time in the tumor and lymph nodes will be derived from serial scintigrams . Scintigrams will be acquired in a Siemens dual-head ECAM gamma camera equipped with ultra-high resolution collimators. This combination will be able to achieve a system resolution of 7mm FWHM (tested with Tc-99m at a distance of 10 cm). One transmission scan will be performed before injection. Then, whole-body and planar imaging will continue at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Geometric-mean images will be used to derive the Ga-67 concentration in the tumor and in the lymph node and be correlated with volumes from anatomic imaging including MRI.

5.3.1 Urine and Blood Collection.

For the Ga-67 GIMA group, patients will be asked to provide urine samples at the following time intervals: before injection and during scintigraphy sessions at 2, 4, 24 hours and one of the 2nd, 3rd or 4th day after injection. Patients will be instructed and provided containers to collect all urine output up to the last day of imaging and urine samples will be collected during the scintigraphy sessions. The patients will be provided with urinals and urine containers that at marked with time information and are encouraged to adhere to the time marking by using the correct container and to

return the containers during the nuclear imaging sessions. Urine samples will have volume, time of excretion, and radioiodine content measured. After measurement, urine samples will be stored behind lead bricks for decay for at least a week. It will be disposed of after the radiation level falls to background level- this will occur within a few days because of the relatively low activity as a small fraction excreted from the 0.2 mCi total dose. All these patients will be asked to provide blood samples (2-5 cc each) at nuclear imaging sessions to characterize the systemic Ga-67 clearance and whole-body and organ radiation dosimetry. Blood samples will be collected by a physician, nurse or nuclear medicine technologist. Aseptic technique will be used and care taken to avoid infection and discomfort. If occurs, fainting spells will be monitored through observation and vital sign measurement until they resolves..

5.3.2 Ultrasonography

The injectate volumes will be monitored using ultrasonography using parameters for routine breast imaging, following each MRI session. Our animal experiments indicate that using the routine clinical ultrasound instrument, GIMA was detectable with the implanted tumors of 3 cm in diameter. Therefore, the patients will be studied with routine clinical parameters (10-12 MHz or 5 MHz with harmonics in a Seimens Anteres clinical ultrasound imaging devices) after MRI. Harmonics will be applied also to decrease potential artifacts. Since ultrasound measurements are relatively operator-dependent, to avoid biases ultrasound images will not be acquired or interpreted by the radiologist who performs the injection under MRI or interprets the MRI.

5.3.3 F-18 FDG PET

The patient will have had a baseline F-18 FDG PET scan as part of the clinical work-up to evaluate the tumor glucose metabolism before entering this protocol. Immediately after the last nuclear follow-up scan of Ga-67 GIMA, the patient will undergo another F-18 FDG PET scan. About 10 to 5 mCi F-18 FDG will be injected intravenously followed by a period of 45 minutes of uptake and then scanning for about 30 minutes for the whole body. The F-18 PET scan will be evaluated qualitatively for uptake in the breast tumor and semi-quantative using the established standard uptake values (SUV's).

5.4 Dosimetry modeling of beta and gamma emissions from radionuclides

There are three components necessary for the estimation of absorbed doses to tissues surrounding the injected activity: 1) The energy deposited in the surrounding tissues will be determined using radiation transport analysis [21], 2) the geometry of the activity distribution (source region) will be determined using MR image data, and 3) the total number of radioactive transitions that occur in the region will be determined using data from the scintigram. Both beta and gamma emissions will be evaluated. The total radiation absorbed doses will be derived for the tumor and surrounding

tissues.

The volumetric data measured from MRI will be used to derive the S-values of the tumors using voxel-based simulation [22] to calculate the radiation absorbed doses to the injection sites and the surrounding tissues. Radiation dose rates, or S-values, will be compared with those from the sphere [18] and shell models [19] to evaluate the effects of potentially heterogenously distributed injectate in the tumor. Such comparison will establish the boundaries of the models and aid choices of dosimetric methods in future studies.

5.5 Pathologic and Autoradiographic Evaluation

Histopathologic data will be collected from the surgical specimen obtained during the scheduled tumor resection (about 7-14 days from injection). If present, the histologic changes from radiation effects [23, 24] in and around the tumor/lymph nodes will be correlated with predicted and measured dosimetry. Selected sections of the tumor with GIMA, as evidence by the light brownish color of deposits, will be frozen and placed on autoradiographic films to develop overnight. Then these tissue sections will be returned to histopathology service to continue tissue processing. No tissue will be retained by our group.

5.6 Potential Radiation Effects and Radiation Safety to the personnel

Although we believe that the radiation involve is low, it should be noted that any amounts of radiation may increase the chance of getting new tumors and radiation from the GIMA may affect the tumor cells. With a biologic half-life of 30 hours and effective half-life of 21 hours, the residual Ga-67 GIMA will be less than 2% of the original dose after the 5 days of imaging (or 6 effective lives). At 7 days after injection, there will be essentially negligible residual amount (2 micro Ci, in total) of Ga-67 and health risk is minimal to health personnel, as long as general body-fluid precaution is followed including washing hands.

6.0 Pretreatment evaluation

Select Section Title: 6.0 Pretreatment evaluation

Prior to the imaging procedures, subjects will be questioned to obtain a medical history, and given a complete physical examination including a mammogram, breast MRI and laboratory tests including CBC to determine eligibility. Patients who have not previously received a breast MRI (with contrast if necessary) and F-18 FDG PET will have one of each performed (at the cost of the study) prior to entry into the study to evaluate the tumor size and assure absence of tumor necrosis. Women with child-bearing potential will receive a pregnancy test.

7.0 Evaluation During Study

Select Section Title: 7.0 Evaluation During Study

Immediately before and up to the 4th day after injection with Ga-67 GIMA, blood samples and urine collection will be taken from the patients at each of the nuclear imaging sessions (2, 4, 24 hours and one of the 2nd, 3rd or 4th day) to measure radiogallium clearance and retention.A F-18 FDG PET will be performed on the same day after the last nuclear imaging and PET.

8.0 Evaluation of Toxicity

Select Section Title: 8.0 Evaluation of Toxicity

8.1 Toxicity

The radiation absorbed doses to the body and organs are low for both the low doses (0.2 mCi, maximum) of Ga-67, compared with the routine dose of 8 mCi of Ga-67 citrate for tumor localization studies. The residual of 1 mg of Fe in the tissue is also not expect to present significant toxicity, considering the routine intramuscular injection of up to 1000 mg of iron sulfate for the treatment of anemia.

8.2 Stopping Rule

End-point will be defined as grade III/IV toxicity (NCI criteria, Appendix L) in 2 or more of the 5 patients in a particular dose level.

9.0 Criteria for Removal from the Study

Select Section Title: 9.0 Criteria for Removal from the Study

9.1 All patients will be followed with reasonable efforts until 2 months after injection. Any patient initially accepted into the study, but who subsequently is determined to be ineligible for radionuclide evaluation will be removed from the study. The reason and time of removal will be documented.

9.2 The development of unacceptable toxicity is defined as unexpected, irreversible or grade 4 toxicity.

9.3 Non-compliance by patient with protocol requirements.

9.4 Patients have the right to withdraw from the study at any time without consequence. If a patient withdraws from the study, reasonable attempts will be made to document the reason for withdrawal.

9.5 Any patient can be removed at the discretion of the investigator or sponsor.

10.0 Number of Patients

Select Section Title: 10.0 Number of Patients

This is a study of biodistribution. One groups of 5 patients will be studied with 0.2 mCi Ga-67 GIMA.

10.1 Only descriptive statistics (mean, variance, ratios and diagrams) will be applied to analyz e the results of dispersed volumes by MRI and ultrasound, tumor and organ percentage of injected doses, dosimetry, potential toxicity and histologic changes.

10.2 The volumes by MRI and ultrasound, and percentage of injected radiation in the tumor and organs as determined by PET/CT will be collected and sent to our outside consultant Dr. Rick Sparks (creative Developments, Inc, Dosimetry Service, Knoxville, TN) for computation of the dosimetry profiles. Because of the limited number of 5 patients, only descriptive statistics will be applied to analyze the data. The raw data will be stored until both this and the companion Ga-68 GIMA study are completed and then will be disposed of. The raw data will be kept under lock and key in our files for at least 5 years from the onset of this study.

11.0 Reporting Requirements

11.0 Reporting Requirements

Select Section Title: 11.0 Reporting Requirements

11.1 Adverse Event Reporting to USAMRMC

Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study, and all volunteer deaths should be promptly reported by telephone (301-619-2165), by email (hsrrb@amedd.army.mil), or by facsimile (301-619-7803) to the Army Surgeon General's Human Subjects Research Review Board. A complete written report should follow the initial telephone call. In addition to the methods above, the complete report can be sent to the U.S Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

11.2 Procedures for Reporting Serious Adverse Events

Serious and/or unexpected adverse events are submitted in writing to the M.D. Anderson Cancer Center Institutional Office of Protocol Research (OPR) within 10 working days of the adverse experience. Unexpected fatal or life-threatening experiences are phoned immediately to the Office of Protocol Research (713-792-2933). A follow up written report is submitted to OPR within 10 working days. The form for communication to OPR of all serious adverse events is appended to this plan.

For all protocols conducted at M.D. Anderson Cancer Center, the Principal Investigator is responsible for submitting adverse event reports to the Institutional IRB and the HSRRB and/or USAMRMC, Office of Research Protections on an ongoing basis. Adverse event reports are submitted to the Institutional Office of Protocol Research (OPR), where they are entered into

PDMS and forwarded to the designated IRB vice chairperson for review. Attached to each adverse event report is a listing of all prior adverse events submitted for that protocol. Any comments, questions or changes the IRB requests to the protocol as a result of this review are conveyed to the principal investigator. The investigator response and protocol modification process is monitored by the IRB vice-chairperson and OPR support staff. The vice chairperson presents the report on adverse event review to the full committee at the next IRB meeting.

An adverse event report compilation is provided once annually to the M.D. Anderson Cancer Center IRB. Comments, questions or other considerations from the IRB are conveyed to the principal investigator for evaluation, discussion and implementation.

11.3 Reporting of Subject Death

The death of any subject during the study or within 30 days of study completion (as defined above), regardless of the cause, must be reported within 24 hours by telephone, to the principal investigator and/or study coordinator and the HSRRB and/or USAMRMC, Office of Research Protections. A full written report must follow as soon as possible. If an autopsy is performed, the report must be provided to the Sponsor (MD Anderson Cancer Center).

Reports of all **serious adverse events, including deaths,** must be communicated to the appropriate Institutional Review Board or ethical review committee and/or reported in accordance with local law and regulations.

11.4 Other reports to DOD HSRRB.

- All amendments will be approved by the MD Anderson Cancer Center Clinical Research Compliance and the HSRRB prior to implementation. If there are deviation from the protocol, the deviation will be reported to the medical monitor and MDACC IRB for approval. Deviation that effects patient safety or integrity of the study will be reported to the DOD HSRRB.

12.0 References

Select Section Title: 12.0 References

1. Vaidya JS, Baum M., Clinical and biological implications of the Milan breast conservation trials. Eur J Cancer Jul;34(8):1143-4, 1998.

2. Edmundson GK. Vicini FA. Chen PY. Mitchell C. Martinez AA. Dosimetric characteristics of the MammoSite RTS, a new breast brachytherapy applicator. Int. J. of Radiat. Oncol. Biol. Physics. 52(4):1132-9, 2002.

3. Nag S, Kuske RR, Cicini FA, Arthur DW and Wwicker RD. Brachytherapy in the

treatment of breast cancer. Oncology (Hungtinton). 15(2): 195-202, 2001.

4. King TA, Bolston JS, Kuske RR, Fuhrman GM, Scroggins TG and Jiang XZ Long-term results of wide-field brachytherapy as the sole method of radiation therapy after segmental mastectomy for T(is, 1,2) breast cancer. Amer. J. Surg. 180(4):299-304, 2000.

5. Vicini FA, Baglan KL, Kestin LL, Mitchell C, Chen PY, Frazier RC et al. Accelerated treatment of breast cancer. J. Clin. Oncol. 19(7):1993, 2001.

6. deGuzman AF, Karvelis SKC, Shaw EG, et al. Brachytherapy Of Re-Resected Malignant Glioma Cavity Margins Using An Inflatable Balloon Catheter And Liquid 125I Source: A Phase I Study. J.Nucl.Med. 41(5 Suppl): 274P, 2000.

7. Dempsey JF, Williams JA, Stubbs JB, Patrick TJ and Williamson JF. Dosimetric properties of a novel brachytherapy ballon applicator for the treatment of malignant brain-tumor resection-cavity margins. Int. J. Radiat. Oncol. Biol. Phys. 42(2): 421-429, 1998.

8. Johnson N. Soot L. Nelson J. Franzini MD. Vea H. Gruner S. Kulawiak L. Young K. Sentinel node biopsy and internal mammary lymphatic mapping in breast cancer. American Journal of Surgery. 179(5):386-8, 2000.

9. Doting MH. Jansen L. Nieweg OE. Piers DA. Tiebosch AT. Koops HS. Rutgers EJ. Kroon BB. Peterse JL. Olmos RA. de Vries J. Lymphatic mapping with intralesional tracer administration in breast carcinoma patients. Cancer. 88(11):2546-52, 2000.

10. Bergqvist L, Strand SE, Persson B, et al. Dosimetry in lymphoscintigraphy of Tc-99m antimony sulfide colloid. J Nucl Med 1982; 23:698-705.

11. Glass EC, Basinski JE, Krasne DL, et al. Radiation safety considerations for sentinel node techniques. Ann Surg Oncol 1999; 6:10-11.

12. Hung JC, Wiseman GA, Wahner HW, et al. Filtered technetioum-99m-sulfur colloid evaluated for lymphoscintigraphy. J Nucl Med 1995; 356:1895-1901.

13. Kim, E. E., Wong, F. C. Radiation Dosimetry and Safety in Lymphoscintigraphy. *In:* K. Hunt, M. Ross (eds), The Mastery of Lymphatic Mapping. New York. Springer-Verlag, (in press).

14. Loevinger R, Budinger TF, Watson EE. MIRD Primer. Society of Nuclear Medicine, 1991.

15. Evans JF. Stabin MG. Stubbs JB. Specific absorbed fractions of energy from internal photon sources in brain tumor and cerebrospinal fluid. Medical Physics. 22(3):331-40, 1995.

16. Ferrari M, Cremonesi M, Bartolomei M, Stabin M, Agosteo S, chinol M, et al. Dosimetric Modeling for locoregional treatment of brain tumors with 99-Y-Biotin. Eur. J. Nucl. Med.27(8):925, 2000.

17. Sparks, R.B., Crowe, E. A., Wong, F. C., Toohey, R. E., Siegel, J.A. Radiation dose distributions in normal tissue due to adjacent tumors containing I-131 or 90Y: J Nucl Med. 43(5):91P, 2002, Suppl.

18. Wong, F.C., Kim, E. E., Sparks, R., Wong, W-H, Yang, D.J. and Podoloff, D.A. Three Sphere Dosimetry Models (0.4 cc, 2cc and 10cc) of 12 Commonly Available Radionuclides for Locoregional Therapy or Extravasation. J. Nucl. Med. 42:243P, 2001.

19. Wong, F.C., Sparks, R., Kim, E. E., Yang, D. J., Podoloff, D. A. Simulation of radiation dosimetry in intra-cavitary injection of eight radionuclides in five shell models. J Nucl Med, 43(5):90P, 2002, Suppl.

20. Colombetti LG, Goodwin DA, Togami E. Ga-68-labeled macroaggregates for lung studies. J. Nucl. Med. 11(12): 704-707,1970

21. MCNP manual, Monte Carlo N-Particle Transport Code System. RSIC 1994.

22. Yoriyaz H, Stabin MG, dos Santos A.Monte Carlo. MCNP-4B-based absorbed dose distribution estimates for patient-specific dosimetry. J Nucl Med. 42(4):662-9, 2001

23. McCormick B, Strom E., Craighead PS, et al, Radiation Therapy Oncology Group. Research Plan 2002-2006. Breast Cancer Working Group. Int. J. of Radiation Concol, Bilol, Physics. 51(3Suppl 2):56-7, 2001.

24. Mirza AN, Fornage BD, Sneige N, uerer HM, Newman LA, Ames FC and Singletary SE. Radiofrequency ablation of solid tumors. Cancer J. 7(2):95-102, 2001.

THE UNIVERSITY OF TEXAS
MD ANDERSON
CANCER CENTER

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Radiation Dosimetry from Intratumoral Injection of Radionuclides into Human Breast Cancer Ga 67 GIMA
2005-0219

Subtitle: Intratumoral Injection of Gallium 67 GIMA for Gamma Camera Imaging and Magnetic Resonance Imaging

1. _____ _____
Participant's Name I.D. Number

You are being asked to take part in this clinical research study at The University of Texas M.D. Anderson Cancer Center (hereinafter referred to as "UTMDACC" or "the institution"). This research study is strictly voluntary. This consent form explains why we are performing this research study and what your role will be if you choose to participate. This form also describes the possible risks connected with being in this study. After reviewing this information with the person responsible for your enrollment, you should know enough to be able to make an informed decision on whether you want to participate in the study. This study complies with all laws and regulations that apply.

You are being asked to take part in this study because you have breast cancer and you have a surgery scheduled to remove the cancer.

DESCRIPTION OF RESEARCH

2. PURPOSE OF STUDY

The goal of this clinical research study is to learn how special radioactive molecules called gallium-iron macroaggregates (GIMA) distribute (travel and spread) in the body after they are injected into breast cancer tissue. This is not a therapeutic trial and you will not benefit from your participation.

3. DESCRIPTION OF RESEARCH

For the common diagnostic procedure called lymphoscintigraphy, small radioactive particles (called colloids) are injected into a lymph node. Then, a special nuclear medicine scanner is used to watch these particles slowly distribute in the body. Researchers noticed the larger the molecule, the slower the particles move in the body. In fact, some of the larger colloid molecules seem to stay at the site of injection with very little movement. Since the amount of radiation on these colloids is very low, they cannot be used to treat cancer. New molecules have been developed called GIMA. These GIMA molecules have been designed to act like the colloid molecules (very slow-moving). However, the GIMA molecules were made to carry larger radioactive particles. Since these molecules are very slow-moving, they can be injected directly into a tumor without spreading out very far from the site of injection. In this way, radiation can be delivered directly to the tumor tissue without spreading to normal tissue.

For this study, small particles of radiation (Ga-67) will be attached to GIMA molecules. These molecules will then be injected into the breast cancer tumor. Special scanners will be used (gamma camera and MRI) to "see" how far the molecules move away from the injection site. If it is found that the molecules do not move very far, in the future, more radiation particles can be attached to the GIMA molecules. These GIMA molecules can then be used to treat cancer with radiation. The radiation level in GIMA (but not its size) can be measured from outside of the body by a gamma camera. The amount of iron in GIMA can be measured from outside of the body by a MRI scanner. The distribution of iron in GIMA can be used to determine the size of the GIMA collection by the appearance of darkening in the images around the injection site. It will be injected by a MRI-compatible needle to avoid potential injury during MRI. The small amounts of iron (one thousandth of a gram) in the GIMA injection will not be sufficient to cause physicial movement of the particles becase the particles are firmly dispersed within the tumor tissues. Ultrasound measurement is non-invasive and may provide similar size information and may in fact prove to be a more convenient method to monitor the size of GIMA in the future. Combining the radiation level and size measurements will allow researchers to determine how much radiation is delivered to the tumor and to other organs (if any).

A total of 5 people with breast cancer will be recruited in this protocol. Participants will receive a GIMA with a particle of radiation attached (Ga-67) that gives off a lower amount of radiation (0.2 mCi). The amount of radiation you will receive from the GIMA molecules is very low, about the same as a routine nuclear medicine scan (or 2-3 Chest X-rays).

The GIMA will be injected directly into the tumor tissue with the help of a MRI scanner. If you prefer to have local anethesia, you will receive about 1cc of lidocaine at the needle entry site above the tumor. If you have allergy or previous

adverse reaction to lidocaine, another local anethestics will be applied. A 20-gauge 7cm long needle that is compatible with MRI facilities will be used to inject the GIMA. The MRI scanner will be used to make sure the needle is inserted directly into the tumor. Also, MRI scans will be done within one hour after the injection. An ultrasound examination of the injection site will also be performed to confirm the location of the GIMA particles

You will undergo nuclear imaging scans in the nuclear medicine clinic at 2, and 4 hours after the injection as well as 24 hours, and one of the following 3 days. You will also have MRI scans and ultrasound following each of these nuclear imaging sessions. You will be asked to have blood samples (1 teaspoon) and urine samples collected during each nuclear imaging session. Blood samples will be obtained by our nurses or physician or nuclear medicine technologist. This study also requires collection of all your urine output at different time points from before injection to the last imaging session. Urinals as well as time-marked containers will be provided to you from our clinic staff. It is important to use the correctly marked containers and return them during the nuclear imaging sessions. The physicians, nurses and technologist will assure of your comfort during the imaging sessions and you may freely express your wishes to avoid discomfort during the imaging sessions. If your require ear plugs during MRI's, they will be provided to you.

After the last nuclear imaging procedure, you will have a F-18 FDG PET scan to measure the use of glucose in your breast tumor. This is a procedure you already had before entering this study and involve injection of a small amount of radioactive sugar.

There has been no known toxicity in human use for the FDG PET and the additional radiation is in the range of 1 to 2 chest X-rays.

Participants will have their regularly scheduled surgery. The tissue that is collected during the surgical procedure will also be studied by the pathologist doctor to see whether there is effect of this investigation on the tumor tissue. Part of the tissue collected will be retained temporarily (1 to 2 days) for analysis of the small amount of radioactivity left before the tissue is returned to the pathologist for routine analyses of tumor tissues and subsequent disposal. No tumor tissue will be retained specifically for this investigational study. Participation into this study will not delay your surgical schedule which is scheduled between 7-14 days from the injection of GIMA.

This is an investigational study. The research human use of radioactive Ga-67 GIMA has been approved by the UTMDACC Radiation Drug Research Committee which has been authorized by the FDA. The GIMA, nuclear imaging scans, ultrasound examinations and MRI scans performed for this study will be provided free of charge. Because participation in the Ga-67 GIMA group requires additional trips to UTMDACC over 5 days, you may be partially reimbursed for lodging expenses. If you live more than 50 miles form UTMDACC, and choose to stay in a

local hotel during the study, you may be partially reimbursed up to 4 nights (up to $70 per night). Up to 5 participants will take part in this Ga-67 GIMA portion of the research and up to a total of 5 participants will take part in this study. All will be enrolled at UTMDACC.

This protocol is partially funded by a research grant from United States Army Medical Research and Materiel Command. It should be noted that representatives of the U.S. Army Medical Research and Materiel Command and the Food and Drug Administration (FDA)are eligible to review research records as a part of their responsibility to protect human subjects in research. Other than medical care that may be provided and any other payment specifically stated in the consent form, there is no other compensation available for your participation in this study.

4. RISKS, SIDE EFFECTS, AND DISCOMFORTS TO PARTICIPANTS

While on this study, you are at risk for the side effects listed in this form. You should discuss these with the study doctor or your regular doctor. The known side effects are listed in this form, but they will vary from person to person. Many side effects go away shortly after the study drug is stopped, but in some cases, side effects may be serious, long lasting, and/or permanent and may even cause death.

Giving GIMA through the needle into the breast cancer tumor may result in pain at the injection site and or infection. GIMA have radiation particles attached to them. Radiation may increase the chance of developing new cancer. The radiation may also alter the cells in the tumor causing changes for the future planning of further treatment.

MRI (Magnetic Resonance Imaging) uses a large magnet instead of x-rays to take pictures of the inside of your body. People who have metal in their bodies (pacemakers, neurostimulators, certain clips, or staples from prior surgery) may not receive a MRI. The magnetic field used in MRI scanning may harm such people or cause problems with devices such as pacemakers. Part or all of the body will be passed into a long, narrow tube (scanner) which is open at both ends. The scanner has an intercom, which will allow you to speak to the doctors and staff during the procedure. The machine will produce a loud knocking noise. This is normal. You will be given earplugs to protect your ears. In addition, you may feel light vibrations throughout your body. Some people, especially those who have a tendency to feel uncomfortable in small or closed spaces, may feel “closed in” and become anxious while in the scanner. If you feel ill or anxious during scanning, doctors and the MRI staff will give comfort or the scanning will be stopped.

Ultrasound examination involves no ionizing radiation and is a safe routine procedure to evaluate breast tissues. In this study, it is used to confirm and follow the gross distribution of GIMA particles; while MRI is used to follow the refined disibution of GIMA.

Gamma camera scan is a medical technique that externally monitors the radioactivity in body and will be used to track the movement of the Ga-67 GIMA through the body. The gamma camera can take pictures of Ga-67 GIMA and "see" where it is in the body. By watching how Ga-67 GIMA travels through the body and studying where Ga-67 GIMA collects, researchers can learn if any radiation is deposited in certain organs in the body. Some people may feel “closed in” while lying in the scanner. However, the scanner is open at both ends and an intercom allows you to talk with doctors and staff. If you feel ill or anxious during scanning, doctors and/or technicians will give comfort or the scanning will be stopped.

For this study, the Ga-67 GIMA is radioactive substances of low radiation levels. The total amount of radiation you receive from this study is about the same as 2-3 chest x-rays.

You may experience pain, bleeding, and/or bruising from the blood draws. You may faint and/or develop an infection with redness and irritation of the vein at the site where blood is drawn. These infections (if present) may delay your scheduled surgical procedure.

This research study may involve unpredictable risks to the participants.

If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinical free of charge. You will only be treated for injuries that are directly cause bvy the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questionals about this medical care, talk to the prinicpal investigator for this study (Dr. Franklin C. Wong, 713-794-4649) or the Protocol chairman (Dr. Gary Whitman, 713-745-3520). If you pay out-of-pocket for medical care elsewhere for injuries cuaised by this research, contact the principal investigator. If the issue cannot be resolved, contact the U.S.Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at 301-610-7663/2211.

5. POTENTIAL BENEFITS

If this research study shows that GIMA stays at the injection site, future cancer therapies may be developed. This information may be of benefit to future patients. There are no benefits for you in this study.

6. ALTERNATE PROCEDURES OR TREATMENTS

You may choose not to take part in this study, participate in a different research study or go directly to surgery without participating in any other study.

I understand that the following statements about this study are true:

7. According to the institutional conflict of interest policy, the principal investigator of this study and my primary physician cannot have a financial interest in any aspect of this research. However, in instances of medical emergency, it is possible that I may be cared for by a physician and/or administrator who has some form of financial interest in the sponsor of this study.

As of 06/10/2003, the following investigators on this study have disclosed an equity or stock option interest in the sponsor of this study: Through the University of Texas M. D. Anderson Cancer Center, Dr. Franklin C. Wong, a collaborator of this protocol has filed a patent application to the U.S. Patent and Trademark Office on radionulcide cancer therapies including the method of producing carrier-free GIMA. For these reasons, there is potential conflict of financial interest (intellectual properties) in this study involving Dr. Franklin C. Wong, The University of Texas, and UTMDACC. Dr. Franklin C. Wong is also the principal investigator of a U.S. Army Breast Cancer Research Grant supporting this study. Dr. Gary Whitman is the principal investigator who will supervise this study in UTMDACC. Either Dr. Gary Whitman or Dr. Mark Dryden will perform the injection of Ga-67 GIMA while you are in the MRI scanner.

The University of Texas M.D. Anderson Cancer Center has a financial interest in the sponsor of this study.

The University of Texas System has a financial interest in the sponsor of this study.

8. If I want to receive updated information regarding the financial interests of any physician and/or administrator at UTMDACC who has cared for me, I may call the Conflict of Interest Coordinator at (713) 792-3220. Upon request, I will be given access to information disclosing the identity of all physicians and/or administrators who have a financial interest in the sponsor of this study.

9. My participation is voluntary.

10. I may ask any questions I have about this study, including financial considerations, of my treating physician. I may contact the principal investigator for this study Dr. Gary Whitman at 713-745-3520 or the Chairman of the institution's Surveillance Committee at 713-792-2933 with any questions that have to do with this study.

11. I may withdraw at any time without any penalty or loss of benefits. I should first discuss leaving the study with my physician. Should I withdraw from this study, I may still be treated at UTMDACC.

12. I understand that the study may be changed or stopped at any time by my doctor, the principal investigator, the study sponsor, or the Surveillance Committee of

UTMDACC.

13. I will be informed of any new findings that might affect my willingness to continue participating in the study.

14. The institution will take appropriate steps to keep my personal information private. However, there is no guarantee of absolute privacy. The Food and Drug Administration ("FDA"),and/or United States Army Medical Research and Materiel Command might review my record to collect data or to see that the research is being done safely and correctly. Under certain circumstances, the FDA could be required to review the names of participants.

15. You will no be responsible for research-related costs. If I suffer injury as a direct result of participation in this study, the institution will provide reasonable medical care. I understand that I will not receive reimbursement of expenses or financial compensation from the institution, the sponsor, the investigators or the United States Army Medical Research and Materiel Command for this injury. If you are hurt or get sick because of this research study, you can receive medical care at an Army hospital or clinic free of charge. You will only be treated for injuries that are directly caused by the research study. The Army will not pay for your transportation to and from the hospital or clinic. If you have questions about this medical care, talk to the principal investigator for this study, (insert name and telephone number of principal investigator). If you pay out-of-pocket for medical care elsewhere for injuries caused by this research study, contact the principal investigator. If the issue cannot be resolved, contact the U. S. Army Medical Research and Materiel Command (USAMRMC) Office of the Staff Judge Advocate (legal office) at (301) 619-7663/2221. for this injury. I may also contact the Chairman of UTMDACC's Surveillance Committee at 713-792-2933 with questions about study related injuries.

16. Unless otherwise stated in this consent form, all of the costs linked with this study, which are not covered by other payers (HMO, Health Insurance company, etc.), will be my responsibility.

17. I recognize that there are no plans to provide any compensation to me for any patents or discoveries that may result from my participation in this research.

Authorization for Use and Disclosure of Protected Health Information

A. During the course of this study, the research team at UTMDACC will be collecting information about you that they may share with the FDA and/or United States Army Medical Research and Materiel Command. This information may include your treatment schedule and the results of any tests, therapies, or procedures that you undergo for this study. The purpose of collecting and sharing this information is to learn about how the treatment affects your disease and any side effects you experience as a result of your treatment.

Your doctor and the research team may share study information with certain individuals. These individuals may include representatives of the FDA and/or the above listed sponsor, clinical study monitors who verify the accuracy of the information, individuals with medical backgrounds who determine the effect that the treatment has on your disease, and/or individuals who put all the study information together in report form. The UTMDACC research team may provide this information to the FDA and/or the above listed sponsor at any time.

B. There is no expiration date for the use of this information as stated in this authorization. You may withdraw your authorization to share this information at any time in writing. More information on how to do this can be found in the UTMDACC Notice of Privacy Practices (NPP). You may contact the Office of Protocol Research at 713-792-2933 with questions about how to find the NPP.

C. If you refuse to provide your authorization to disclose this protected health information, you will not be able to participate in the research project.

D. I understand that my personal health information will be protected according to state and federal law. However, there is no guarantee that my information will remain confidential, and may be re-disclosed at some point.

CONSENT/AUTHORIZATION

Having read and understood the above, and having had the chance to ask questions about this study and reflect and consult with others, I give _____ permission to enroll me on this study. I have been given a copy of this consent.

_____ _____
SIGNATURE OF PARTICIPANT DATE

_____
TYPED OR PRINTED NAME OF PARTICIPANT

_____
ADDRESS OF PARTICIPANT

_____ _____
WITNESS OTHER THAN PHYSICIAN OR INVESTIGATOR DATE

_____ _____
SIGNATURE OF PERSON RESPONSIBLE & RELATIONSHIP DATE

_____
TYPED OR PRINTED NAME OF PERSON RESPONSIBLE

I have discussed this clinical research study with the participant and/or his or her authorized representative, using a language that is understandable and appropriate. I believe that I have fully informed this participant of the nature of this study and its possible benefits and risks and that the participant understood this explanation.

_____ _____
SIGNATURE OF STUDY DOCTOR OR PERSON OBTAINING CONSENT DATE

Animal Care and Use Form
University of Texas M. D. Anderson Cancer Center

ACUF Protocol # 06-04-05871
(Temporary Identification Number: FW-20EV-0420P)

Have you had a previously IACUC approved ACUF on this work? ○ Yes ● No

I. Investigator and Proposal

A. Principal Investigator:	Franklin Wong
PI Title:	Associate Professor
PI Phone:	713-794-4649
PI Department:	Nuclear Medicine
PI Fax:	713-794-5456
PI Unit:	59
B. Contact Person:	Sharon I Davis
Contact Title:	Administrative Assistant
Contact Phone:	713-794-4647
C. Study Location:	Houston
D. Document Details	
Version:	**07**
Version Status:	**Administratively Approved and Activated 04/17/2006**
Save Status:	**Saved as "Final"**
Re-Submitted By:	Resubmitted by: Franklin Wong -- 4/11/2006 1:21:48 PM
ACUF Admin Action:	Accepted By: Lydia G. Jackson -- 4/17/2006 1:56:50 PM

E. Proposal Title:
Image-Monitoring of Locoregional Radionuclide Therapy

F. Describe the goal(s) of this research project in lay terms:
1. To evaluate the distribution of particulate and soluble radiopharmaceuticals in lung and prostate tumors in a large animal model using MRI.
2. To characterize the rate of loculation or spread of radiopharmaceutical after injection in tumors and normal tissues using nuclear imaging instruments including gamma-camera or PET measurement.
3. To evaluate the effects on tumor and other organs after injection with radiopharmaceuticals by measuring the metabolic rates using PET.
4. To compare the distribution and loculation of radiopharmaceuticals after injecting into prostate cancers and lung cancers.
5. To derive the radiation doses to the tumor and to different organs by combining the information from the MRI and nuclear imaging, so as to project to future human doses.
6. To evaluate the histologic changes in tumors after intratumoral injection of radionuclides

G. Who will perform experimental manipulations on the animals?

1. Kamran Ahrar; MD; Assistant Professor; YEARS EXPERIENCE WITH SPECIES: 3 Training form on file; Will perform surgery

2. Dvms Personnel; Various; Various; YEARS EXPERIENCE WITH SPECIES: Various; Will administer anesthesia

3. Sacrif Veterinary Staff; Various; Various; YEARS EXPERIENCE WITH SPECIES: Various; Will administer anesthesia

Will Any personnel need training or assistance in surgical procedures, aseptic technique or postsurgical care?	○ Yes ● No

H. List All Collaborators (include all individuals other than those directly involved with the animal Manipulations)

1. Kenneth Wright; Diagnostic Radiology; CONTRIBUTION TO PROJECT: Provide facility, guidance and mentoring

2. Jason Stafford; Physics; CONTRIBUTION TO PROJECT: Provide support for MRI scanning of the animals

3. Dawn Cavanaugh; Physics; CONTRIBUTION TO PROJECT: CT image Quality Analysis

4. Osama R Mawlawi; Imaging Physics; CONTRIBUTION TO PROJECT: PET imaging

I. Do the studies proposed within this ACUF involve the use of industry-sponsored research? (e.g. sponsored research agreements with pharmaceutical or biotechnology companies)
○ Yes ● No

II. Animal Model

Description of Animals

A. Species: Dog

B. Stock/Strain: Mongrel
Beagle

Trans/KO Gene:

Do any of these strains develop unique pathological conditions?
○ Yes ● No ○ Unknown

C. Sex: Both

D. Age: Adult

E. Weight: 15-60kg

F. Why is it necessary to use animals in this project?

Preclinical information from animal models is necessary before proceeding to clinical trials
Cell culture or mathematical models cannot simulate host-tumor interactions involved in metastasis (or angiogenesis)
We have developed a tumor model in the lungs and in the prostate in dogs.

G. Why is this species used?

Large tumors comparable to human tumors can be developed in the canine model, suitable for development and refinement of imaging techniques and/or targeted therapy
FDA recommended model

H. Total number of animals requested over a three year period.

Note: ACUFs are approved for a 3 year period.

Number to be purchased:	20
Number to be bred on site:	0
Current Inventory of animals from previous protocol:	0
Total Number Request:	**20**

I. Why is this number of animals required?

We are investigating the distribution of radiopharmaceuticals in tumor grafts in 2 different locations- each study will take 9 animals to reach statistical significance. Small allowance will be given to the small rate (10%) with which the tumor will not grow.

J. Can in vitro systems or other approaches, e.g. mathematical models, be used to reduce the number of animals in this project? ○ Yes ● No

Why can other methods not be used to minimize the number of animals used?

In vitro studies do not take into account the dynamic physiological events that take place in a live animal. Blood circulation has significant impact on the extent of radiopharmaceutical distribution in the lungs or prostate and on the radioaction dosimetry. The only effective method of evaluating these devices, is to simulate a patient with a live animal model. Gross mathematical models have been performed but need to be refined and cannot substitute for various effects of blood flow and geometry. In vitro studies would not simulate a lung tumor or prostate in a live human being.

III. Animal Housing and Nutrition

A. Type of Animal Facility:

Conventional:
large animals, rabbits, guinea pigs, hamsters, chickens, frogs, and some rodents
Radioactive:
animals which are to receive radioisotopes

B. Animals will be housed:

Clinical Research Building:
dogs, swine, monkeys, rabbits

C. Primary animal enclosures housing(cage, run, stall, pasture)

Conventional:
large animals, rabbits, guinea pigs, hamsters, chickens, frogs, rodents

D. Animal Feed

Conventional:
large animals, rabbits, guinea pigs, hamsters, chickens, frogs, and some rats and mice

E. Drinking Water

Conventional:
large animals, all non-SPF animals, unless otherwise specified.

IV. Agents Used In Animal

HAZARDOUS AGENTS: include carcinogenic chemicals, antineoplastic drugs, infectious microbial agents, viral agents, toxins, recombinant DNA. Do not include anesthetics or routine antibiotics

Note: Grants, programs, projects, etc., involving the use of hazardous agents are reviewed by the Institutional Biosafety Committee. Contact the Office of Research Administration (713-563-3879) to determine the appropriate method of approval for pilot projects involving hazardous agents' use in animals.

Note: In your flow chart(s), please include an informational description (e.g. dosage, routes of administration, frequency, durations of exposure, etc.) of agents to be used in this research protocol.

A. Will Hazardous Agents Be Used? ● Yes ○ No

1. Indicate Hazardous Agents to be used below:
Hazardous Agents must be reviewed by the Institutional Biosafety Committee (IBC).
Submit a copy of your approval letter to the IACUC Office (required for approval)
Please update your IBC to include this protocol Number

Chemical Agents:

1. Cyclosporine - -- IBC Approval #: 0904-103-HA-1

B. Will Human or Animal tissues or cells be injected or transplanted as part of this study?
● Yes ○ No
Will Cells or Tissue be genetically modified before use in animals? ○ Yes ● No

Animal Tissues or Cells Types:

1. Type: cTVT; Species of origin: Dog; Source: SCID Mice

C. Will Radioactive Agents Be Used? ● Yes ○ No
Submit a copy of your approval letter to the IACUC Office (required for approval)
Please update your RSC to include this protocol Number

Radioactive Agents:

1. Ga-68 GIMA -- RSC Approval #: 1062

2. F-18 FDG -- RSC Approval #: 1062

3. Ga-67 citrate or chloride -- RSC Approval #: 1062

4. In-111 Chloride -- RSC Approval #: 1062

D. Will External Radiation be administered to animals? ○ Yes ● No

E. Will Non-Hazardous Experimental Agents be used? ● Yes ○ No
List all non-hazardous agents that will be used. Provide information on flowsheet

Non-Hazardous Agents:

1. Radiographic contrasts for CT and for MRI

2. Sodium Iodide solution (0.5 - 5.0 iodide mg/ ml in saline) in 0.5-2 ml for interstitial injection. This is a nonradioactive drug used for protection of thyroid from radioactive iodine.

3. Iron (ferric) chloride (1mg Fe in 1ml saline or water) for IM injection to provide MRI/CT signal

4. Gadolinium (Gd) chloride (1mg Gd in 1ml of saline or water) for IM injection to provide MRI/CT signal

F. During administration of any of the above agents, animals will be:

☒ **Anesthetized/Unconscious**
☐ **Unanesthetized/Conscious**

V. Experimental Procedures

A. Type of Restraint

1. Will restraint of animal be necessary? ● Yes ○ No
Answer "Yes" if using any degree of restraint. The housing of animals in standard cages is not deemed restraint.

Indicate type of restraint, and the maximum time any one animal would be restrained within a 24 hour period.

Restraint Type:	Duration:	Dosage Information:
☒ **Physical**	Less than 1 minute	
☒ **Chemical**	Less than 90 minutes	buprenex - 0.01mg pentothal - to effect isoflurane - 1.5-3.0%

2. Will paralytic drugs be used without associated general anesthetic? ○ Yes ● No

B. Anesthesia

If Anesthetics/analgesics/sedatives are used, include complete dosage information.
NOTE: This information should also be provided in Flow Sheet, Section VII, for each experimental group.

1. Will anesthesia be used for any reason? ● Yes ○ No

Anesthetic	Dose	Route
1) buprenex	0.01mg/kg	i.m.
2) pentothal	to effect	i.v.
3) isoflurane	1.5-3%	Endotracheal tube
4)		
5)		

2. Indicate what methods will be used to monitor anesthetic depth

Measure Respiratory Rate
Measure Body Temperature
Measure Heart Rate

3. Building and Room Number where animal(s) will be anesthetized: Clinical Research Building TB.4262

C. Analgesia

Note: For information about the regulations/policies concerning the use of analgesia, please consult the *IACUC's Analgesia Standard Operating Procedure* .

1a. Moribund animals must be euthanized-In the event that an animal associated with this protocol experiences pain or suffering (e.g. after major survival surgery), analgesics will be given.

Euthanized
Treated with appropriate anesthetics/analgesics/tranquilizers after consultation with veterinary staff

2. Will you use other techniques to minimize experimental pain or distress? ● Yes ○ No

Techniques:

- Other:
  Observation to ensure uneventful recovery from anesthesia. Post-procedural analgesics (buprenex): will be for 3 days, then as determined is necessary by DVMS vet.
  Building and Room Number: Research Clinical Building

D. Surgery

1. Will there be any surgical manipulations of these animals? ● Yes ○ No

Include in the Flow Sheet, Section VII, a detailed textual description of the surgical technique. Include descriptions of surgical site preparation, incision, all surgical manipulations, and closure technique (e.g., suture material, clips).

2. Surgery will be performed in:

Other
Building and Room Number:
Tan Zone Basement, Computed Tomography, TB.3848

3. The surgical manipulations will result in animal: ● Survival ○ Nonsurvival

Survival Surgery Catagories **(Aseptic technique and appropriate analgesia is required for all survival surgeries)**
Major Survival Surgery - surgical interventions that penetrate a body cavity or have the potential for producing a permanent handicap in a recovering animal

IACUC's Analgesia Standard Operating Procedure

4. Will there be multiple survival surgery required? ○ Yes ● No

5. What post surgical analgesic care or therapy will be used?
Routine observation post anesthesia. The surgery required is CT guided needle placement in lung and is considered minimally invasive. Recovery from it is quick.

Analgesic	Dose	Route
1)		
2)		
3)		

6. What other postsurgical care or therapy will be used?
(e.g. heat lamps, etc.)

E. Sample collection from living animals

1. Will you be collecting tissues from animals? ○ Yes ● No

F. Other Information

1. Will adjuvant be used? ○ Yes ● No
http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual

2. Will food and/or water be restricted for reasons other than a normal fast (</= to 12 hrs) associated with surgery/anesthesia? If Yes, please provide the reason and length of time food and /or water will be restricted. ○ Yes ● No
http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual

3. Will the mouse ascites method be used for monoclonal antibody (MAB) production?
○ Yes ● No
http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual

4. Will any animal manipulations not previously mentioned be performed? ○ Yes ● No

5. Are there any postmortem procedures? ● Yes ○ No

Please describe the procedures:
Necropsy and harvest of tissues form the chest including lungs and mediastinum and pleura and/or prostate glands and pelvic tissues. Tissues will be evaluated by gross and histopathology

6. Will animals be removed from the DVMS/DVS facilities for any experimental procedure?
● Yes ○ No

Indicate building and room number where the procedure will be performed.
Research MRI Facility, RB.2625
LightSpeed-16 scanner is in the Green Zone on the third floor (G3.3585a)

G. Monitoring of Animals

1. Describe any physical or physiological impairment of animals resulting from experimental manipulations (e.g., MTD50, neoplasia). If tumor(s) exist, state the maximum size, burden, and length of time the tumor will be present. Scientific justification must be provided if requesting total tumor burdens greater than 1.5cm diameter in mice and 2.0cm diameter in rats.
The IACUC policy on tumor burdens in animals is available on the IACUC Website at:
http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual

All animals will be inoculated with cTVT for development of lung tumors and or prostate to a maximum size of 4 cm. A desirable tumor size for treatment is 2 to 3 cm. Based on our previous experience with this model, it will take 6 to 8 weeks following inoculation to achieve this goal. All treatment and follow up should be completed in 1 week. The maximum lenght of time anticipated to complete the project in each animal is 10 weeks.

2. Describe monitoring procedure/schedule, including weekends and holidays, for morbid and moribund animals.*

Animals will be monitored daily.

3. Describe criteria to determine morbidity, and the point at which moribund animals will receive euthanasia.*

The following criteria will be assessed for detection of moribund animals. Lack or loss of appetite will be investigated and correctable causes will be addressed. Those animals that continue to have marked diminished appetite leading to emaciation and dehydration will be considered moribund. Lack or decreased activity level will be evaluated, and those animals with persistent and prolonged decreased mobility will be considered moribund. Respiratory status will be assessed, and those animals that show signs of respiratory distress despite adequate measures to correct the underlying problem will be considered moribund. Persistent urinary or bowel obstruction (from prostate tumor) will be considered moribund

*__NOTE:__ All investigators are expected to continue to monitor animals at least daily, including weekends and holidays. Morbid is defined as affected with disease or illness; moribund is defined as being in the state of dying.

H. Euthanasia

Include age and euthanasia method for unused rodent pups, if applicable.
(Ether and chloroform are not approved agents for euthanasia because of potential flammable, toxic and carcinogenic hazards)

Note: The use of hypothermia to induce anesthesia in rodent pups < 6 days old requires the use of an acceptable method of euthanasia and must be scientifically justified.
If applicable, please include justification below:

1. Indicate the method(s) to be used:

Exsanguination with Anesthesia

2. Will death be used as an endpoint? ○ Yes ● No
http://utm-int01a.mdacc.tmc.edu/dept/prot/orahomepage.nsf/IACUC%20Manual

VI. Flow Sheet

Immunosuppresion: Immunosuppression therapy will begin 1-4 days prior to tumor inoculation. Each dog will be immunosuppressed with 10 mg/kg cyclosporin b.i.d. for 2 weeks and then s.i.d. until the end of the study. Cyclosporin will be administered by mouth (po).

Anesthesia: For all imaging and interventional procedures, sedation is induced by an i.m. injection of buprenex (0.3 mg) and a s.c. of atropine (0.04 mg/kg). Pentothal will then be given i.v. to effect and an endotreacheal tube will be inserted. Anesthesia will be maintained with Isoflurane (1.5-3%) and oxygen.

Tumor Inoculation: With lung tumor grafts, the animal is placed in a CT scanner, and the right and left lateral chest walls are shaved and prepared for aseptic surgery. CT scans of the lungs are obtained without and with intravenous injection of contrast medium (meglumine-diatrizoate; 35 ml). At least 2 sites will be selected for a single inoculation each. The site will be shaved, the skin will be prepared in sterile fashion using betadine. Under direct CT guidance, a 22 gauge Chiba needle is placed in a preselected location and 0.5 ml of freshly harvested and prepared canine sarcoma tumor fragments are injected into the lung parenchyma. Following inoculation, CT scans of the lungs is repeated without contrast injection. The animal is then allowed to recover from anesthesia.
For prostate tumor grafting, a ventral midline laparotomy will be performed to expose the prostate, followed by injection of the tumors (0.5 ml total) in the anteroventral aspects of both lobes and the prostate will be surgically fixated under the anterior wall of the bladder for future image-guided injection of radionuclides. The animal is then allowed to recover from anesthesia.
The tumor will be monitored with MRI and/or CT and/or ultrasounds without constrast.

Additional CT images are obtained using a clinical CT scanner located on the 3rd floor of the green zone as well as a volumetric CT scanner located in the basement of Tan zone. These CT scans will be performed on different days and will require anesthesia on separate occasions. These images will be obtained once a week with and without contrast starting at 2 weeks after inoculation to monitor the growth of the tumor. The transport route to the diagnostic CT scanner in the green zone is included in the attached file.

Transport route:

T1 to B1, via the north end lifts & overhead door
B1 Gimbel corridor to Y1 elevators (8, 9, 10) to 3rd floor.
Y3 corridor through patient waiting room to G3 corridor
G3.corridor across bridge to G3.CT facility
See the attached map of the route along the 3rd floor corridor.

Transport route to G3 CT&PET.

When tumors reach approximately 2 cm in size, the animals are treated as follows.

Pre-injection Imaging: At lest one day prior to radionuclide injection, each animal undergoes non-contrast CT, MRI and F-18 FDG (1-5mCi) baseline PET scan to determine the metabolic status of the tumor. The MRI will include diffusion weighted images that are particularly sensitive to acute and subacute ischemic changes.

The dogs are transported from TB animal facility along the 1st floor corridor hallway (Blue Zone)
to the Yellow Zone as indicated in the enclosed Transport route.

Route Map to Signa 1.5T.p

Radionuclide injection, MRI and PET/CT: Approximately 1 mCi of Ga-68 GIMA (half-life of 1 hour) or 2 mCi of F-18 FDG (with 3 mg FeCl3 or 5 mg of Magnevist, a MRI contrast agent, and/ or 10mg iodine equivalent in Omnipaque, a CT contrast agent) will be injected intratumorally, under MRI and/or ultrasound (for the prostate group) guidance. MRI will be then used to determine the initial physical distribution of the particulate drug Ga-68 GIMA (from the Iron) or soluble drug F-18 FDG (from the co-injected Fe Cl3 or Magnevist or CT contrast). PET/CT scanning will be used to determine the radioactivity distribution and decay of Ga-68 GIMA or F-18 FDG in the injectate over the next 1-2 hours. A follow-up MRI session will be performed to evaluate the potential dispersion of the injectate

Follow-up FDG-PET: Whole-body FDG PET scan using 1-5 mCi of F-18 FDG intravenously will be performed to compare the metabolic status of the tumor.

Following completion of the follow-up FDG-PET scan, an optional procedure may be performed to study movement of solutes after interstitial injection. Nonradioactive sodium iodide (0.5- 5mg iodide /ml in saline) in 0.5- 2ml volumes with or without 0.2-1 mCi of F-18 FDG will be injected under CT guidance into the lungs in the PET/CT suite. Serial PET/CT will be performed over 1-1.5 hours to obtained the spatial and temporal clearance patterns of F-18 FDG using sodium iodide as the CT contrast for better spatial resolution.

PET scanning will be performed during the weekend or afterhours in the clinical PET/CT scanners when no patient is on schedule.

No animal will undergo more than 3 consecutive days of imaging. This is not likley to occur because of the short half-lives of the radiopharmaceuticals.

Necropsy and Pathology: Following the last MRI scan, each animal is sacrificed by exsanguination under deep anesthesia with isoflurane. The lung and/or prostate tissues are harvested for gross and histopathology evaluation. The effectiveness of treatment is assessed by light microscopic evaluation of the treated lung tumors.

dog flow chart-FW-2.p

Aug 17, 2005: This ACUF is approved to perform survival surgery to implant TVT tumors in the lungs and prostate of dogs in up to 4 sites; to perform multiple MR and PET/CT imaging procedures using radioisotopes and standard contrast agents; and to perform percutaneous intratumor injections of radioisotopes + various carrier agents. We currently have a dog that has undergone tumor implantation, imaging, and intratumoral treatment. The tumors were treated under MR and CT guidance on Mon, Aug 15. The dog is scheduled for another imaging session (MR and PET/CT) on Friday. Due to technical difficulties on Monday, the therapy injections missed the target (the tumors) in both sides of the lungs - the prostate injections went OK. We are now requesting to repeat the pulmonary tumor injections on Friday, Aug 19, 2005 during the regularly scheduled imaging session. We will perform the

procedures exactly as already approved in the protocol; no additional anesthesia or imaging is required; basically, the only additional procedure is to perform the percutaneous lung injection. We are requesting this addition on this one dog, one time only.

Modification 4-11-06: Shortly before or after the CT/MRI evaluation, a nuclear imaging procedure will be undertaken on selected groups of animals (2-3, based on logistics) for confirmatory studies of interstitial locuation of radionuclides after intramuscular (IM) injection. This will be conducted while the animal is already under anesthesia for a previously approved procedure, to eliminate the need for additional anethesia procedures. The animal will then be transported to YB.5774 to receive intramuscular injection in the leg muscle of 0.5 mCi of Ga-67 chloride or In-111 chloride (with or without 1mg of Fe chloride or Gd chloride for MRI signals). Nuclear imaging will be performed using an animal gamma camera Siemes M-CAM to obtain static 5-10 minute images. Subsequently, the animal will undergo serial similar gamma imaging during subsequent anethesia sessions for other planned CT, MR or PET. Radiation protection procedures including daily swipe test and radiation exposure measurement will be conducted until after necrosy during which the injected muscule(s) will be preserved for at least 3 more weeks before histopathologic evaluation.
Please note that these procedures will only be conducted in connection with a previously approved imaging study, and the extent of the additional manipulation will be an intramuscular injection followed by approximately 15 minutes of imaging. There are no procedures will represent significant additional pain or distress to the animal.
Radiation safety approval for use of these agents in the dog has been received. Existing transport routes are appropriate for these additional procedures and isotopes.

VII. Addenda

USDA Addendum:

USDA Category "C"

☐ This project will not involve pain or distress to animals, and therefore, no pain relieving drugs are needed.

USDA Category "D"

☒ This study involves accompanying pain or distress to animals. However, appropriate anesthetic, analgesic or tranquilizing drugs will be used*

* List procedures, drugs, volumes, doses, route, and duration of anesthetic, analgesic, or tranquilizing drugs in Section VII, flow sheet, for each experimental group.

USDA Category "E"

☐ This study involves accompanying pain or distress to animals without the use of an appropriate anesthetic, analgesic, or tranquilizing drugs. **

** Attach a complete explanation of the reasons why drugs for relieving pain or distress were not used. For example, explain how and/or why drugs would adversely affect the test/study results.

USDA Responses to Animal Act Regulations

Response to Animal Act Regulations Part 2, Subpart C (Research Facilities), #2.31 IACUC,

[d] Reviews of activities involving animals
[1] [ii] P.I. has considered alternatives to procedures that may cause more than momentary or slight pain or distress to the animals, and has provided a written narrative description of the methods and sources (e.g. Animal Welfare Information Center) used to determine that alternatives were not available.

[iii] P.I. has provided written assurance that the activities do not unnecessarily duplicate previous experiments.

Part [ii]

Response to part [ii]: Select the appropriate choice below

☐ No pain, discomfort, or suffering is involved.
☒ Alternatives to procedures that may cause more than momentary or slight pain or distress to animals were not found.
☐ Alternatives to procedures that may cause more than momentary or slight pain or distress to animals were found.

Part [iii]

Literature Search Details:
20 relevant references were found for the last 9 years. The UTMDACC Research Library resource personnel and Medline and Pubmed computer-assisted literature reviews (e.g. AWIC, Agricola, Medline) were used to search for information about alternatives.

List the top three searches:

Date:	Search terms included
07/19/2004	Intratumoral and radionuclide and prostate and animal model
07/19/2004	Intratumoral and radionuclide and lung and animal mode
07/19/2004	dosimetry and intratumoral and animal model

The Alternative(s) found did not satisfy my research requirements for the following reasons:

Intratumoral unsealed radionuclide injection is a novel treatment for lung tumors and prostate cancer. There is very little data on safety, effectiveness and complications of the intervention. Despite some antedoctal clinical reports in humans, to date there is no studies done to evaluate the distribution and dosimetry of radionuclides after intratumoral injection in large animals.

Response to Part [iii]:

● There are no activities involving animals that duplicate previous experiments.
○ Replications of some activities involving animals are necessary.

VIII. Funding Sources

Source	Status
NIH/NCI	Pending

Investigators are responsible for budgeting sufficient funds for animal purchase and maintenance. Information on the current maintenance charges is available on the DVMS Intranet Website.

IX. Investigator's Assurance Statement

Principal Investigator:
I accept and will conform to all Federal and State laws and guidelines, and all institutional policies and procedures concerning the care and use of animals in research, teaching, or testing. I also assure that I and all persons named on this form will complete the institutional animal care and use training program and submit documentation before working with animals. I understand that I have a responsibility to notify in writing the Institutional Animal Care and Use Committee of any changes in the proposed project or personnel, relative to this application, prior to proceeding with any animal use, and will provide an annual project status report.

Principal Investigator Signature: _____

Principal Investigator Name: Franklin Wong Date: _____

Chairman/Division Head:
I have reviewed this request for animal care and use and have found the proposed research to be scientifically meritorious.

Chairman/Division Head Signature: _____

Chairman/Division Head Name: Donald Podoloff Date: _____

Lydia G.
Jackson/MDACC@MDACC
06/30/2006 11:14 AM

To Franklin Wong/RAD@RAD
cc
bcc
Subject Annual Review

The University of Texas M.D. Anderson Cancer Center

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE (IACUC) CONTINUING REVIEW APPROVAL

Meeting Date: October 25, 2005

Protocol Number:	06-04-05871
Protocol Approval Date:	OCT 21,2004
Protocol Title:	Image-Monitoring of Locoregional Radionuclide Therapy
Investigator:	Wong, Franklin C., M.D., Ph.D., J.D. Department of Nuclear Medicine Unit 59

The Institutional Animal Care and Use Committee did a yearly review of the above named and numbered protocol. The IACUC has granted continuing approval of your protocol. For information concerning this protocol, please contact your appropriate IACUC Coordinator:

Thank You!

Lydia Jackson
IACUC Coordinator
713-563-3888

MD ANDERSON CANCER CENTER
MODIFICATION REQUEST FOR ANIMAL CARE AND USE COMMITTEE
HOUSTON CAMPUS

Date Received 04/11/2006

To: IACUC
From: Franklin Wong/RAD

ACUF ID #: 06-04-05871
PI Name: Franklin Wong
Version: 07
Subject: ACUF Resubmission Cover Memo for '06-04-05871'

Did the IACUC Analyst request this modification for grant compliance? No

THIS BLOCK FOR THE ANIMAL CARE AND USE COMMITTEE ONLY

Meeting Date: May 16, 2006	Pre-Reviewer: K. Naff
Recommendation: Administrative Approval	Pre-Review Date: 4/15/06

Modification Requests:

Add edit right, Dr. Borne
Add read rights, Dr. Chickerneo
Add radioisotopes X 2
Add nonhazardous agents X 2
Add nuclear imaging procedure

Date Approved: April 15, 2006

If adding animals, please include explicit justification and indicate appropriate statistical analyses have been conducted to determine an appropriate number for the proposed study. The total number of animal numbers requested should equal the total number represented in accompanying diagrammatic flow sheets.

Modification Requests (please include flow sheets* or anesthesia & surgery pages if applicable)**
When adding a strain, include a justification for adding:

Revision # 1
Section and Item: Access to document
Change Made: add editor Dr. Agatha Borne and add reader Dr. Farrah Chickerneo
Rationale: provide access for better support

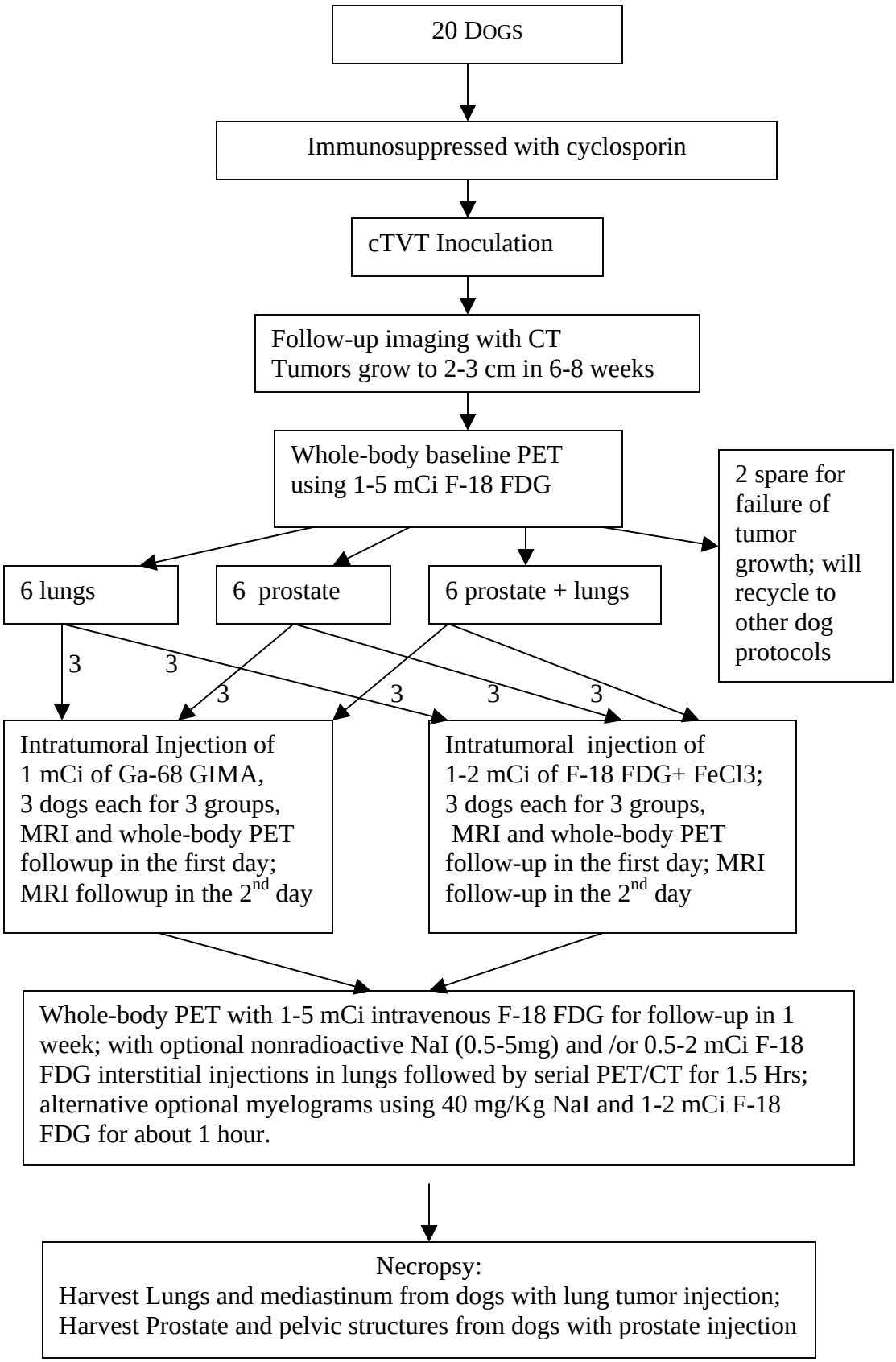
Revision # 2
Section and Item: IV-C radioisotopes
Change Made: add description of Ga-67 chloride or citrate (an imaging agent for human cancer), and In-111 chloride (an imaging agent for human bone marrow)
Rationale: One of the goals of this research is to characterize the rate of loculation or spread of radiopharmaceutical after injection in tumors and normal tissues using nuclear imaging instruments including gamma-camera or PET measurement. We have developed information using the rat model for IM localization and dwell time for these agents. We now want to confirm these findings by performing these additional minor procedures using our existing dog model to evaluate intramuscular localization of these isotopes. Radiation safety approval for use of these agents in the dog has been received. Existing transport routes are appropriate for these additional procedures and isotopes.

Revision # 3
Section and Item: IV-E
Change Made: add non-hazadous agents: Iron chloride (1mg/ml) and Gadolinium chloride (1mg/ml) to provide MRI/ct signals for the intramuscular injectates.
Rationale: These compounds will produce a signal on the MRI/Ct scan that will serve to localize intramuscular injection.

Revision # 4
Section and Item: VI Flowsheet description, before transport routes
Change Made: add description of Ga67 or In-111 imaging procedure

Shortly before or after the CT/MRI evaluation, a nuclear imaging procedure will be undertaken on selected groups of animals (2-3, based on logistics) for confirmatory studies of interstitial locuation of radionuclides after intramuscular (IM) injection. This will be conducted while the animal is already under anesthesia for a previously approved procedure, to eliminate the need for additional anethesia procedures. The animal will then be transported to YB.5774 to receive intramuscular injection in the leg muscle of 0.5 mCi of Ga-67 chloride or In-111 chloride (with or without 1mg of Fe chloride or Gd chloride for MRI signals). Nuclear imaging will be performed using an animal gamma camera Siemes M-CAM to obtain static 5-10 minute images. Subsequently, the animal will undergo serial similar gamma imaging during subsequent anethesia sessions for other planned CT, MR or PET. Radiation protection procedures including daily swipe test and radiation exposure measurement will be conducted until after necrosy during which the injected muscule(s) will be preserved for at least 3 more weeks before histopathologic evaluation.
Please note that these procedures will only be conducted in connection with a previously approved imaging study, and the extent of the additional manipulation will be an intramuscular injection followed by approximately 15 minutes of imaging. There are no procedures will represent significant additional pain or distress to the animal.
Radiation safety approval for use of these agents in the dog has been received. Existing transport routes are appropriate for these additional procedures and isotopes.

Rationale: add description of optional procedure, no additional anethesia needed


20 DOGS
Immunosuppressed with cyclosporin
cTVT Inoculation
Follow-up imaging with CT
Tumors grow to 2-3 cm in 6-8 weeks
Whole-body baseline PET
using 1-5 mCi F-18 FDG
2 spare for failure of tumor growth; will recycle to other dog protocols
6 lungs
6 prostate
6 prostate + lungs
3
3
3
3
3
3
Intratumoral Injection of
1 mCi of Ga-68 GIMA,
3 dogs each for 3 groups,
MRI and whole-body PET
followup in the first day;
MRI followup in the 2nd day
Intratumoral injection of
1-2 mCi of F-18 FDG+ FeCl3;
3 dogs each for 3 groups,
MRI and whole-body PET
follow-up in the first day; MRI
follow-up in the 2nd day
Whole-body PET with 1-5 mCi intravenous F-18 FDG for follow-up in 1 week; with optional nonradioactive NaI (0.5-5mg) and /or 0.5-2 mCi F-18 FDG interstitial injections in lungs followed by serial PET/CT for 1.5 Hrs; alternative optional myelograms using 40 mg/Kg NaI and 1-2 mCi F-18 FDG for about 1 hour.
Necropsy:
Harvest Lungs and mediastinum from dogs with lung tumor injection;
Harvest Prostate and pelvic structures from dogs with prostate injection

Nuclear Imaging: From Rats to Dogs to Humans

Franklin Chiu-Leung Wong

Department of Nuclear Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX, USA.

The obvious advantages and needs for the use of small and large animals in medical research cannot be over-emphasized. *In vitro* findings from bench-top experiments need to be verified *in vivo* in order to take into account the complex intracellular and extracellular milieu, heterogenous physiological barriers and concurrent biochemical reactions, as well as confounding pharmacodynamic and pharmacokinetic interactions. For practical, as well as economic reasons, *in vivo* verification of *in vitro* findings typically progresses from small animal models to larger animal models and finally to humans. This presentation examines the requirements of each step or stage. Although not purported to be an exhaustive dissertation on each issue of each stage, the scientific, technical and procedural (regulatory compliance) aspects will be discussed for small animal, larger animal and human experiments. Imaging experiments on locoregional radionuclide therapy in our laboratories, using rats and dogs, provide good examples to illustrate these requirements. Finally, based on our own development of imaging studies, the typical linear progression of translational studies will be re-examined to derive an alternative.

Regulatory compliance of animal experiments

Although the use of animals may appear to be a necessity of discovery and a privilege to medical scientists, there are strong and sometimes valid voices from the community against the experimental use of animals. There are also individual concerns about using specific species (such as dogs). The theoretical and ethical issues, as well as regulatory efforts, have been examined and ethical uses of animals are outlined.[1,2] In creating a balance between these issues and the necessity of making advances in medical sciences, the various federal, state and local authorities have passed legislation on the experimental use of animals. Compliance to these regulations is not only necessary. It is the law. Whether animal experiments are performed in large or small institutions, there are federal, state and local regulations, and funding agency as well as institutional policies, governing animal purchase, maintenance, welfare, anesthesia, surgery, recovery, euthanization, necropsy and disposal. In larger institutions, these policies are usually codified in animal protocol forms, which need to be examined and approved by the Institutional Animal Care and Utilization Committee (IACUC). In addition, the use of radioisotopes needs the approval of the Radiation Safety Committee, while the different non-radioactive drugs need the approval of the Institutional Biosafety Committee (e.g. for infectious, toxic or genetic materials). Each of these policies is obvious and necessary. However, concurrent compliance to each policy may be onerous and certainly necessitates earlier filing. It may result in delayed commencement of animal experiments. It took 6–8 months of planning and filing for the approval of each of the protocols involving rats and dogs presented in this article. Additionally, each substantial change in the procedures requires filing and approval of the modification before they can be adapted. Our laboratory sought the collaboration of veterinary medicine staff, starting from the experimental design stage. Their scientific insights and suggestions for procedural shortcuts have proved invaluable to our protocols. While various animal models for human diseases are aptly summarized in other textbooks,[3] this presentation concentrates on the development of experiments from rats to dogs to humans, from the viewpoint of cancer research and, specifically, the field of radionuclide cancer therapy.

The clinical question: locoregional radionuclide cancer therapy

While systemic administration is standard practice for diagnostic nuclear imaging, there are theoretical and practical advantages of locoregional administration of radionuclides for cancer therapy. This later approach is less common, owing to the lack of requisite information on the dispersion, diffusion and dosimetry for locoregional administration to solid tumors. Without these parameters, locoregional use of radionuclides may result in underdose (loss of efficacy) or overdose (toxicity). Animal imaging studies may direct the approach of the human experiments in different manners. It may indicate the appropriateness of the animal model, the need for upscaling or the potential pitfalls. Animal imaging studies may also indicate the inappropriateness of specific animal models. For instance, after a set of initial experiments on rats confirming very rapid clearance of ^{131} I-sodium iodide after intrathecal injection (Figure 1), it was determined that the rat model is not appropriate for predicting human pharmacokinetics because rats have rapid clearance of cerebral spinal fluid at rates about six times that of humans. Other animal studies may be even less likely to produce statistics that will be meaningful for human therapy, and so they are not worth conducting. Without the undue burden of additional animal experimentation, our team has proceeded to conduct two phase I human studies to treat leptomeningeal metastasis and determined the procedure to be safe and efficacious.[4,5] Experience in human intrathecal radionuclide therapy has led our team to the development of intratumoral injection for treatment of solid cancers.

The use of direct application of radionuclides to the tumor bed has advanced to reach the clinical stage, as demonstrated by breast lymphoscintigraphy [6] for the localization of sentinel lymph nodes and by intra-arterial injection of ^{90}Y-labeled particles for the treatment of liver cancers.[7] Typically, intraparenchymal injection of ^{99m}Tc-labeled sulfur colloid particles in the tumor bed will reveal the sentinel lymph nodes as 0.01–0.5% of the particles reach the lymph nodes. The rest of the particulate radiopharmaceutics (i.e. > 99%) disintegrates and deposits radiation around the injection site, albeit small doses from ^{99m}Tc. While the intra-arterial approach of radionuclide cancer therapy is limited by the lack of exact tumor dosimetry and requires technical expertise to access the feeding arteries, intratumoral injection of particulate radionuclide may be an attractive alternative to treat human solid tumors. Although intratumoral injection of particulate radiopharmaceutics has been found to be efficacious in suppressing tumor growth in rat experiments,[8,9] the dispersion and radiation dosimetry need to be evaluated in animals. Radiation-absorbed doses to tumor and to normal organs are important concerns as illustrated by the modeling of ^{131}I and ^{90}Y compounds.[10] Since the dispersion of radiopharmaceutics manifests as changes in volumes on anatomic imaging (MRI), paramagnetic radiopharmaceutics have been developed to assess the intratumoral loculation or dispersion over time and to derive radiation dosimetry profiles.[11] Human radiation dosimetry in the tumors may be derived from imaging of these therapeutic radiopharmaceutics in small animals.

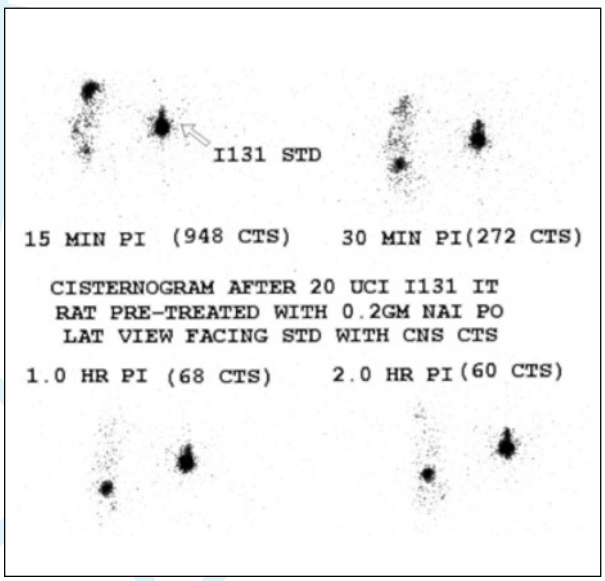


Figure 1. Thirty minutes after intrathecal injection of 20 μCi of ^{131}I-sodium iodide via an indwelling catheter via the foramen magna, the majority of ^{131}I has exited the CSF pathway and returned to the general circulation to be excreted to the urinary bladder. The rapid clearance in the CSF indicated that it is probably not a good model for human studies. Abandonment of this futile rat model led to direct human experimentation in two clinical trials.

Small animal imaging — the rat

The availability of human disease models, including genetic knock-out mice for testing different hypotheses, has driven the development of dedicated small animal imaging instruments for PET, SPECT, CT, MRI and their combinations. Rat tumor imaging is a convenient approach for studying tumor diagnosis and following therapy. After intratumoral injection, ^{67}Ga-labeled iron macroaggregates (^{67}Ga-GIMA) are found largely retained in the breast tumor implants by scintigrams (Figures 2A and B). Based on our Monte Carlo dosimetry models[11] and long residence times (68 hours) in tumors from imaging, the radiation absorbed doses in the tumors ranged from 29 to 143 Gy for 0.2–1 mCi of ^{67}Ga-GIMA, respectively. Indeed, a dose-related response has been reported for ^{67}Ga-GIMA in suppressing tumor growth in rats.[8] The results of these experiments have facilitated our preparation of an ongoing clinical trial to study radiation dosimetry in breast cancer patients, using MRI and SPECT/CT for ^{67}Ga-GIMA as well as PET/CT for ^{68}Ga-GIMA.

Our team has also studied the retention and dispersion of other particulate radiopharmaceutics as well as soluble radiopharmaceutics, including ^{18}F-FDG and ^{18}F-NaI (Figures 3A and B), in rats after intratumoral injection. The retention of ^{18}F correlates with the efficacy in suppressing breast cancer in our rat model. We have derived invaluable parameters for the design of human locoregional cancer therapy.

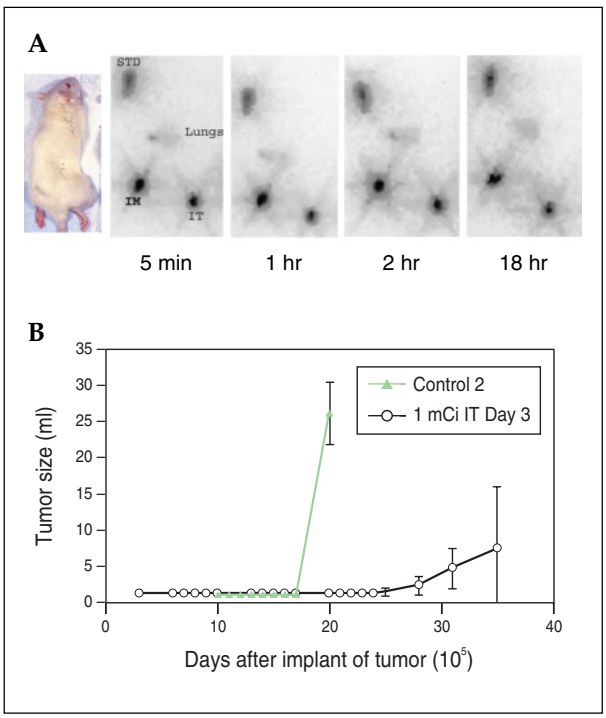


Figure 2. (A) Prolonged retention of GIMA is detected by serial scintigrams after intramuscular (IM) or intratumoral (IT) injection. (B) Tumor suppression is demonstrated by 1 mCi of ^{67}Ga-GIMA after IT injection.

Large animal imaging — the dog

Despite higher costs in animal purchase and maintenance, large animal studies are often necessary to substantiate the findings from small animal models. Large animal studies are more important in imaging studies to approximate the geometric relationships of human organs. Pigs, sheep, horses, monkeys and dogs are commonly used. For instance, our

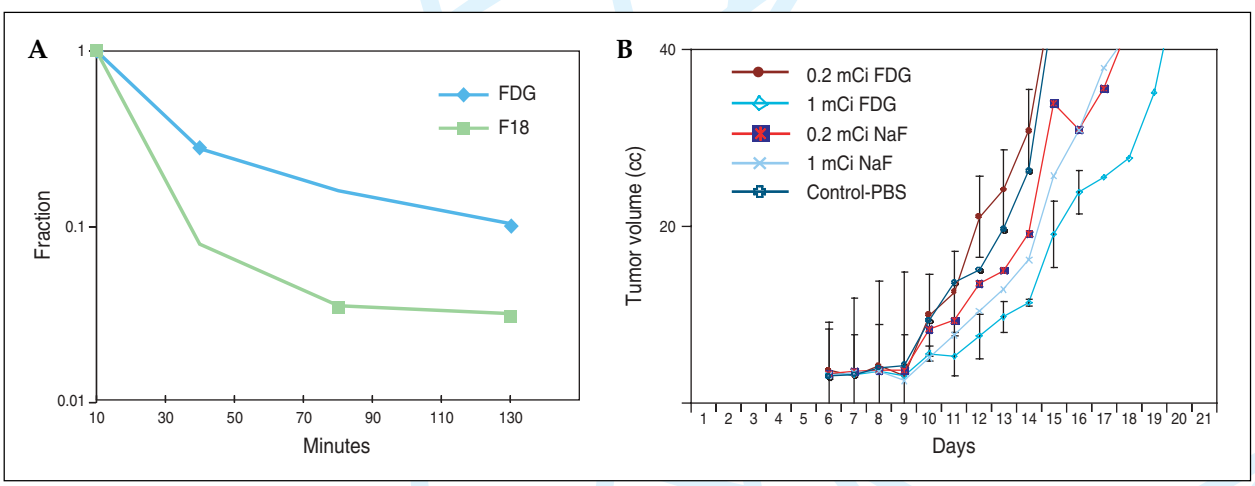


Figure 3. (A) After intratumoral injection, ^{18}F-FDG is retained in the tumors while ^{18}F-NaF exits rapidly. (B) The ability of ^{18}F positrons to suppress tumor growth after locoregional injection correlates with its retention in the tumors.

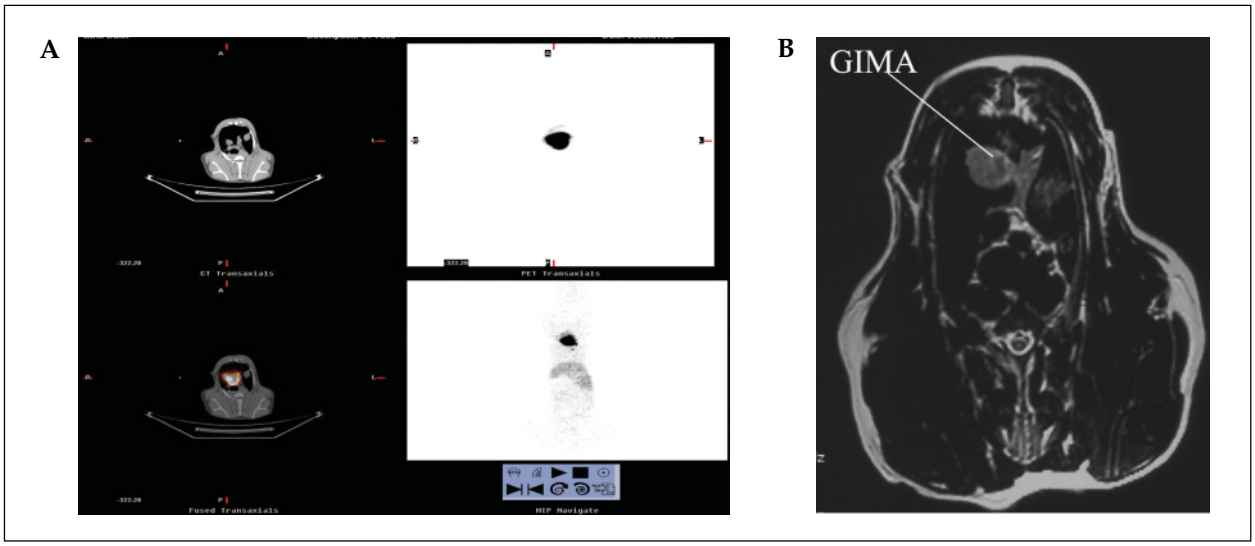


Figure 4. (A) ^{68}Ga-GIMA PET/CT of a dog after injection into lung tumors. (B) MRI detects suppression of gradient resonance echo signal by iron contents inside the tumor where GIMA was injected 4 days earlier.

team has investigated the distribution of GIMA in lung and prostate cancers using dogs because of the availability of tumor lines (canine transmissible venereal tumors [cTVT]) and for convenient animal manipulation. Under MRI guidance, ^{68}Ga-GIMA was injected into tumors implanted into lungs and prostates of dogs. PET/CT and delayed MRI images confirmed the loculation in lung cancer (Figure 4) but leakage in prostate cancer was also noted (Figure 5). Similarly, ^{18}F-FDG is also found to be retained in dog lung tumors with long residence times.

Other than the availability of a disease model in a particular animal species, the choice of specific large animal model depends also on the practical aspects. For instance, some rabbits are more prone to cardiovascular incidents due to stress and may not be a good choice for repeated manipulation. Some monkey species are avoided by animal manipulators because they are more hostile, and other species transmit animal-to-human infections. Nevertheless, our choice of dogs for large animal imaging studies has provided invaluable parameters for our design of therapeutic radiopharmaceutics for clinical trials in the immediate future.

Human studies

Human experimentation inevitably invokes controversies with opposing scientific and ethical issues. It is strictly governed by law in civilized countries and will be conducted only when the likely benefits justifiably outweigh potential risks to research subjects. Even in developed countries like the USA, other factors to consider when conducting human studies are resources available to conduct the experiment and costs to contain unforeseen liabilities. The Human and Health Services oversees administration of the Investigation Device Evaluation (IDE) for new instruments and the Investigational New

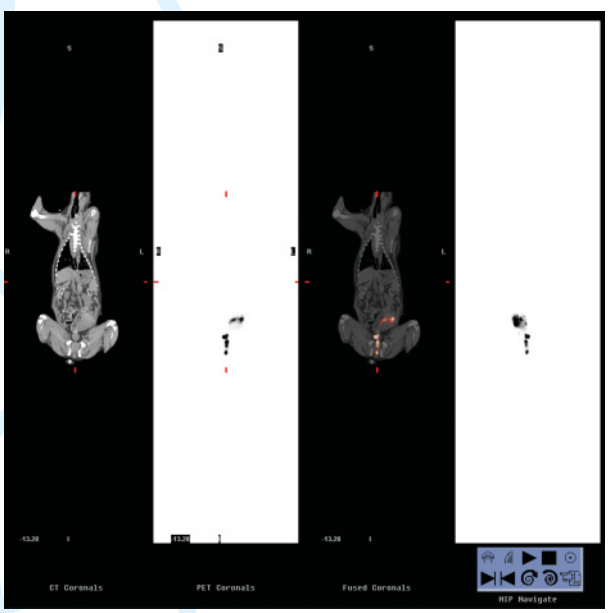


Figure 5. ^{68}Ga PET/CT of a dog after intratumoral injection into prostate cancers. Loculation of ^{68}Ga-GIMA is noted in the prostate; however, there is also leakage into the bladder and urethra.

Drug (IND) oversees administration for new drugs through the Food and Drug Administration (FDA). Almost all new radiopharmaceuticals need to undergo IND processes. FDA approval is required for IND, which usually starts with a Phase I study (to confirm low toxicity) and then proceeds to Phase II (to confirm efficacy) and Phase III (to establish dose range) studies.

Traditionally, Phase I studies are designed to evaluate potential toxicity/untoward effects in humans. The initiation of Phase I studies requires satisfactory demonstration of safety/toxicity profiles from two different animal species. Most of the radiopharmaceutics fulfill these requirements because only minute (submicrogram) quantities of physical materials are involved, which are not sufficient to produce toxicity. In recent years, there is also an emphasis on evaluating immunotoxicities and infectious components of radiopharmaceutics derived from biologic origins. Most countries have similar regulations regarding new radiopharmaceutics to protect the general public. In most USA institutions, human experimentation is governed by the institutional review board (IRB). The IRBs are peer-review groups charged with the responsibilities of initial approval and continuing monitoring of new clinical research activities, including new radiopharmaceutics. Compliance to IRB mandates is not only candid. It is required by law.

A new algorithm for imaging studies in rats, dogs and humans

The conventional linear progression of experiments starts from the test tube to the Petri dish, then to the animals and then to humans. These *in vitro* to *in vivo* translations are lengthy processes with each step consuming months to years. With the current development of sensitive imaging instruments and specific imaging agents in molecular imaging, this linear development algorithm may be greatly shortened. The faithful and specific translation of biologic signals, such as loculation of ^{68}Ga-GIMA or ^{18}F-FDG in rat and dog tumors, has enabled rapid feed-forward and feedback loops during the translation from *in vitro* to *in vivo* stages and from animal to human studies.

As demonstrated by our experience of intrathecal radionuclide therapy over the last 8 years, an unsuccessful rat model (Figure 1) led to its abandonment and direct human experimentation. Insights from the human intrathecal radionuclide therapy led to the development of particulate and soluble radiopharmaceutics for cancer therapy in rats. The rat scintigraphic studies have, in turn, enabled our clinical trial in human breast cancer patients. Development of our imaging studies in the breast cancer clinical trial has stimulated the dog PET/CT and MRI studies of lung and prostate cancers. Our own progress in various small and large animal imaging studies is always directed by clinical needs. It is this type of direct feed-forward and feedback with clinical needs that transforms the conventional linear progression algorithm into a spiral one. In our group, this spiral progression continues and will likely return fruitfully to the therapy of various solid human cancers in the imminent future.

Summary

This is an overview of the development of clinical imaging and small and large animal imaging studies by our team. Compliance to regulations on animal and human studies is mandatory. Modern imaging instruments, including MRI, CT, PET, SPECT and combinations thereof, as well as newer imaging agents, have allowed our team to visualize physiology and anatomy in sensitive and specific manners. These molecular imaging tools have transformed the conventional linear type of therapy development into a spiral type of progression to find solutions to more clinical problems and at faster paces.

Acknowledgements

This work and the clinical trials have been partly supported by the following grants: R21 CA89891 from the US National Institutes of Health, R21 CA97729 from the US National Institutes of Health and BC020808 from the US Dept of Defense, Army Breast Cancer Research Programs.

References

1. Orlans FB. *In the Name of Science — Issues in Responsible Animal Experimentation.* New York: Oxford University Press; 1993.
2. Phillips MT, Sechzer JA. *Animal Research and Ethical Conflict — an Analysis of the Scientific Literature 1966–1986.* New York: Springer-Verlag; 1989.
3. Hau J, Van Hoosier LV, editors. *Handbook of Laboratory Animal Science, Volumes II and III, Animal Models.* Boca Raton, FL: CRC Press; 2003.
4. Wong FC, et al. *J Nucl Med* 2004;45(5 Supp):148P.
5. Wong F. C, et al. *Eur J Nucl Med* 2002;29:S172.
6. Berman CG, Brodsky NJ. In: Khalkhali I, editor. *Lymphoscintigraphy and Lymphatic Mapping. Nuclear Oncology, Diagnosis and Therapy.* Philadelphia, PA: Lippincott, Williams & Wilkins; 2001:297–311.
7. Gray B, et al. *Ann Oncol* 2001;12:1711–1720.
8. Wong FC et al. *Eur J Nucl Med Imaging* 2004;31(2 Suppl): S297.
9. Wong F C, et al. *Euro J Nucl Med Imaging* 2004;3(2 Suppl): S293.
10. Wong FC, et al. *Eur J Nucl Med Imaging* 2004;31(2 Suppl.): S387.
11. Wong FC, et al. *J Nucl Med* 2001;42(5):28.

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EDUCATION & CERTIFICATION

Doctor in Medicine and Surgery, from International University of the Americas, 2003.
General Physician, Costa Rican Board of Physicians and Surgeons, 2004.
Laserscope Certified, 2005 USA.
Radiation Safety Certified from The University of Texas M.D. Anderson Cancer Center, 2006.
Clinical Research Training Program, The University of Texas M. D Anderson Cancer Center, 2006.
Coordinator, Research Data, The University of Texas M.D. Anderson Cancer Center.(Current Position)

QUALIFICATIONS

Radioisotope Use Authorized by The University of Texas M.D Anderson Cancer Center, 2006.
100% Bilingual, English/Spanish - Written and Oral.
Computer Skills: Windows 2000/XP, Word, Excel, Word Perfect, Acrobat and Power Point, basic Internet knowledge
Excellent communication skills.
Demonstrated record of consistent performance and ability to perform different healthcare related procedures.
Collecting and analyzing, records, and maintaining patient information, such as medical history, reports and examination results.

EMPLOYMENT HISTORY

Coordinator, Research Data, The University of Texas M.D Anderson Cancer Center.
Assistant to Primary Care Physician and Laser Clinician as well as Assisting billing and coding in Doctor’s Clinic, Houston, TX
Working with new laser light technology such as green light to promote collagen stimulation including enhanced skin rejuvenation, wrinkle removal, hair reduction, leg vein removal, treatment of different dermatological disorders such as lentigo, rosacea and acne treatments.

On-board Physician (seasonal position) in Lindblad Expeditions, Cruise Ship Sea Voyager, in association with National Geographic.

Traveling with tourists from Costarrican ports to Panama City
Responsible for security, health and well being of approximately 100 people. Taking measures to avoid accidents by going on expedition routs with them and treating small non-life threatening emergencies.

Medical Doctor, Emergency Assistant in Clinic Dr. Marcial Fallas Diaz, San José, Costa Rica.
Working in Emergency Room. Classifying by TRIAGE, with an intake of approximately 200 patients per day. Diagnosing and treating patients with various pathologies or injuries, such as stabbings, gun shot wounds, animal bites, cuts and scrapes, hypertensive urgencies and emergencies, acute myocardial infraction, strokes, non-compensated diabetes patients.

Anatomy Laboratory Teacher in Universidad Hispanoamerica, San José, Costa Rica Managing Anatomy Laboratory and teaching interactive classes with at least 25 students, teaching them basics in anatomy, corps dissections, muscles, arteries, veins and organs.

OTHER RELATED EXPERIENCE
Rotative Internship in different Costarrican Hospitals
Internal Medicine - Saint Vincent of Paul, Class C Hospital - Heredia, Costa Rica.
Taking patient’s medical histories, medical backgrounds, and physical examination. Epidemiology studies in Dengue with the Aedes Aegypti mosquito and the surrounding population, collecting and analyzing, records, and maintaining patient information, such as medical history, reports, and examination results.
Presumptive diagnosis of patients’ pathologies and presenting the patients’ charts to attending specialized physician as well as attending to hospitalized patients and in emergency room and taking on different kinds of medical procedures (invasive and not invasive)

Surgery - Max Peralta, Class A Hospital - Cartago, Costa Rica
Taking patients’ medical histories, medical backgrounds, and physical examinations. Being a part of the cancer research foundation in association with Costarican and Japanese government.

Presumptive diagnosis of patient’s pathologies, presenting the patients’ charts to attending specialized physician and assisting specialized surgeons with different kinds of surgeries including orthopedics, gastrointestinal (specially gastric cancer), thyroid and general surgeries.

Gynecology and Obstetrics - Max Peralta, Class A Hospital Cartago, Costa Rica
Taking patients’ medical histories, medical backgrounds, and physical examinations. Presumptive diagnosis of patients’ pathologies and presenting the patients chart to attending specialized physician.
Assisting specialized surgeons with different kinds of surgeries, such as cesarean sections abdominal hysterectomies, abdominal ultrasound, amniocentesis, cauterizations, complete hysterectomies, chorionic villus sampling, cone biopsies, cryosurgeries, curettage, Episiotomies, LEEP’s, Salpinectomy and others.

OTHER INTERESTS/HOBBIES
Black Belt, I Dan, in Shoto-Kan Karate-Do
Certified by CISKA (Costarrican International Shoto Kan Association).

REFERENCES
Doctor Disha Poonia, Medical Doctor, Family Practice. Phone # 713-686-3700
Brenda Tripp, manager in Doctor’s Clinic of Houston. Phone # 713-981-8185
Other references will be submitted upon request.
Thank you.